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THE RATE OF LIVING

BEING AN ACCOUNT OF SOME EXPERIMENTAL STUDIES ON THE BIOLOGY OF LIFE DURATION

BY

RAYMOND PEARL

THE JOHNS HOPKINS UNIVERSITY

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PREFACE

THE *substance of this book was presented in condensed form as a series of three lectures at University College, London, in June 1927, under the title* "EXPERIMENTAL VITAL STATISTICS." *The material has been chiefly drawn from published and unpublished investigations carried out in my laboratory since 1920. I am indebted to Dr. J. McKeen Cattell for permission to use material previously published in* THE AMERICAN NATURALIST. *To my colleagues, Dr. John Rice Miner, Miss Agnes L. Allen, and Miss Blanche F. Pooler, I am indebted in many ways for help in the preparation of the book.*

RAYMOND PEARL

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THE RATE OF LIVING

CHAPTER I
ORIENTATION

THE study of vital statistics has traditionally been pursued as an adjunct to other matters. It started in the seventeenth century with Captain John Graunt's *Natural and Political Observations mentioned in a following Index, and made upon the Bills of Mortality,* as the bookkeeping department of the public health. Primarily it has always remained just that. William Farr, who firmly placed England in the position of world leadership in biostatistics, which she holds to this day, enormously advanced the significance and respectability of the subject in the minds of those officially concerned with public health administration and hygiene. Since Farr's time an administrative organization for public health concerns is unthinkable without vital statistics as an integral part of its working machinery. The health official depends on his statistician for the evidence to justify his continued existence, as well as for the material which will enable him, by judicious and pertinent propaganda, to make that existence even happier, and hopefully more useful, than it now is.

To all these demands the biostatistician has nobly arisen. Not usually given the credit he deserves, because

of the more spectacular achievements of some of his colleagues, notably the bacteriologists, he has really been the balance wheel of that complex piece of social machinery which now so effectively protects us against the environment, and, in some measure, ourselves. So self-effacing and useful has he been that he has suffered the enormous misfortune of being taken for granted. Nearly everyone has forgotten, or failed ever to think, that the matters with which biostatistics concerns itself constitute some of the most fundamental and important problems of pure biology, and certainly the most neglected ones from anything but the completely practical aspect. The existing structure of the organic world is that of a vast *congeries* of mutually interacting and integrated groups of living things. Its status at any given moment is the resultant of the forces of mortality and natality which exist, and have existed in the past, in all these groups. The most elementary and gentle application of logical thought would lead to the conclusion that it must be impossible to discuss organic evolution intelligently without chiefly talking about birth rates and death rates. But the plain fact is that evolution is now, and always has been, discussed with only the slightest and most casual consideration of these important elements in the case. It is another sad example of the slight influence of logic upon human behavior.

I wish in these lectures to state and defend the thesis that biostatistics, so far from being merely a useful technical handmaiden to preventive medicine, is in fact the

sign, the symbol, and indeed in some respects the very essence, of a *selbständige Wissenschaft,* namely the *biology of groups.* Nothing is more certain today than the fact that in the realm of living things the whole is something different from and more than the mere sum of its individual parts. No amount of knowledge regarding the anatomy, physiology, and psychology of individual termites would enable one to predict that a group of termites would, as a group, construct the kind of nest that it does. The nest is a morphological attribute of the group as a whole, in the same sense that the mouth parts are a morphological attribute of the individual.

The conception that the whole in the organic realm is something more than the sum of the parts has become more generally familiar in recent years, under such labels as *emergent evolution, holism,* and other terms of the same import. Notably to be mentioned in this connection are the writings of Wheeler (132–135), Lloyd Morgan (68), Smuts (128), Roberts (118), Sellars (124), Henderson (47), and Jennings (52).

If the group of living things be considered as group or "whole," it plainly has attributes and qualities which are peculiar to itself. These attributes demand description and measurement, just as much as do corresponding attributes of the individual. Upon them will rest the foundation of any science of group biology. It has been emphasized at various times (82, 83), since the idea was first made plain by the work of Francis Galton that the statistical technique, the methods of modern biometry, furnish

the only means available for accurately and adequately describing and measuring the attributes of groups. " The ordinary methods of description as used in biology fail (i.e., become altogether inadequate) when the attempt is made to deal with any group of individuals, as for example a population, race, variety, species, or larger group. These methods fail because they are fundamentally and necessarily incapable of giving a description of the group (whatever its magnitude) *in terms of anything but the individuals which compose it*. That is to say, they have no way of getting at a description of a group (e.g., a species) as a whole or as such, in terms of its (the group's) attributes and qualities. Let an illustration make this point clear. The purpose of systematic zoölogy is to classify and arrange animals in natural groups. As a necessary step in the carrying out of this purpose it is obliged to attempt to define, which means describe, these groups. But its whole way of going about this process is a confession of the fundamental inadequacy of the method. The systematist frankly makes no attempt whatever to describe or define a particular species as a species (i.e., as a group of animals) in terms of its (the species') qualities. Instead he describes one individual animal belonging to this species; affirms either expressly or tacitly that all other individuals belonging to the species are " about " or " generally " like the individual described, and then calls the net result the definition or description of the species. But now surely this is not a description of the species at all. An adequate description of the species will be one

which takes account of its peculiarities as a unit and indicates how it as a unit or as a whole is distinguished from other similar groups. In making this statement there is, of course, no implication that the facts set forth by the systematist are not desirable and useful. But something more is needed to gain a well-rounded, adequate idea of the group, whether species, variety, or any other."

All that has been said will, I am sure, be fully concurred in by this audience. In fact its repetition here is not unlike the proverbial in-bound coal trade of Newcastle. But the matter can be pushed a little farther. In the general methodology of science Experiment has at least as honorable a position as simple Description. If there is to be a science of group biology it must have its experimental as well as its descriptive side. That the whole philosophy of emergent evolution demands as well as permits this has lately been emphasized by Jennings (52).

In the assertion that there may properly be made such a division of science as group biology, and that it may be studied by the experimental method there is implicated a postulate. This postulate is that rational and experimental determinism exists in the biology of groups just as in the biology of individual organisms. It is not only probable *a priori* that this postulate is true, but the data of group biology support it in fact. If careful attention is given to the regulation of genetic and environmental variables it is possible to repeat the experimental determination of a life table for a particular organism as many times as one desires, and to get always the same result, with as great

precision and regularity as is found in experiments on the physiology of muscle, for example.

Here we come to the specific territory which I am asking you to join me in exploring in these lectures. The point of view regarding experimental biostatistics which I shall try to present has been a natural development. When the department of biometry and vital statistics was organized in the School of Hygiene and Public Health of the Johns Hopkins University an integral part of the plan was the provision of biological laboratory facilities for the *experimental* study of populations, or *groups* of living organisms. The search then began for a suitable animal form with which to do the work. The *desiderata* were: (1) that the animal should be short-lived so that mortality statistics would accrue with reasonable rapidity; (2) that it should breed freely in captivity, so that the statistics of natality should not lag behind those of mortality; (3) that its husbandry in the laboratory should be relatively simple and capable of ready experimental control and modification; and finally (4) that it be a form whose genetics was fairly well known, so that assured purity and homogeneity in this respect could be attained without too great effort or loss of time.

The work first started with mice, which fairly well satisfied these requirements. But at the end of the first year, when we were just ready to start definitive experiments, a devastating calamity wiped out the colony in a half hour. This led involuntarily, but fortunately, to a reconsideration of the entire position, and ultimately to the de-

cision to use in the work the fruit-fly *Drosophila melanogaster* and its mutant forms. Since that time (1919) this has been the organism chiefly studied in my laboratory. It has proven eminently satisfactory.

As has already been suggested a comprehensive study of the biology of groups will involve, at least, the investigation of the following three basic elements in the situation:

a. *Mortality,* that is the qualitative and quantitative aspects of the primary forces tending to bring about the decline or ultimate destruction of groups, involving the investigation of mortality rates, life tables, etc., which are attributes of groups, under diverse conditions in respect of nature and nurture.

b. *Natality,* that is the primary forces of reproduction, tending to bring about the preservation or survival of the group, involving the investigation of rates of fecundity and fertility, which again are group attributes.

c. *The movement of population,* that is the growth or decline of the group as result of the integrated action of the forces of mortality and of natality, involving the investigation of the form and rate of population growth (positive or negative), again under diverse conditions of nature and nurture.

All of these aspects of group biology have been studied experimentally and statistically in my laboratory during the past eight years, and if time permitted it would be a pleasure to review the results which have been obtained in each of these fields. But the limitations of three lectures

make this impossible and I have therefore chosen to confine the discussion mainly to the first topic, mortality. There will necessarily be some references to the other aspects of the matter but these will, on the present occasion, be only incidental.

Up to the time when our work began little had ever been done in any systematic way on the experimental study of the normal duration of life. Curiously enough most of this had made use of *Drosophila* as material. Hyde (49) in 1913 published a paper on the inheritance of longevity in this form. In studying fertility and sterility in different strains of flies he found two strains which differed to a marked degree in respect of length of life, and made crosses to study the behavior of the shortened length of life of the mutant "truncate" in heredity. His numbers were small, but they showed the characteristic increased vigor of first generation hybrids. The shorter average age of the lumped second generation flies indicates that there segregated out in that generation some short-lived flies. His data, however, do not give, or allow one to get, separate averages for the truncate and the normal winged individuals in the second hybrid generation.

His data are summarized in Table 1.

In 1914 Baumberger (2) published data on the length of life of different orders of insects without food, as affected by different constant temperatures, and by exposure to two different temperatures. Since the insects were caught in a net as imagoes the total longevity is not known, so that the results have little significance from the

TABLE I

HYDE'S DATA ON INHERITANCE OF DURATION OF LIFE IN DROSOPHILA

Type of Flies		Inbred Wild	Truncate	F_1	Truncate ♂ X Wild ♀ F_2	Reciprocal F_2	Total
No. of Flies		191	272	42	128	89	722
Mean duration of life in days ...	♂ and ♀	37.4	21.4	47.0	29.5	29.3	....
	♂	40.5	26.9	47.8	32.8	31.1	....
	♀	34.5	18.5	46.4	25.9	27.3	....

standpoint of exact studies. The 359 insects had at 72° F. an average longevity of 4.8 days with a maximum of 15 days; at 62° F. an average of 6 days with a maximum of 23; and at 42° F. an average of 10.9 days with a maximum of 39. For the second part of the experiment 184 larvae of the oak-tree moth were used. The results are too conflicting to allow one to draw any definite conclusions.

In 1915 Liff (62) reported, in his tables, some scattered observations, without discussion, on the duration of life of mated pairs of wild type *Drosophila* and the mutant "pink." The range of longevity exhibited in these tables is from 7 to 63 days, but the work cannot be regarded as a contribution, in even the smallest degree, to the serious study of the problem of life duration.

In 1915 appeared Lutz's (67) paper on natural selection in which he found in each sex a slight negative correlation between the length of adult life and the duration

of the embryonic periods. His distributions for normal length of adult life with varying temperature give the 250 males an average duration of life of 36.3 days, and the 263 females an average of 28.9 days. He also presented distributions in hours of duration of life of flies which were given water but no food, and the correlations of duration of life of these starved flies with wing measurements.

During 1916 and 1917 Loeb and Northrop (63–66) published a series of papers on the effects of food and temperature on duration of life in *Drosophila*. The first preliminary paper in 1916 (63) gave the duration of life of cultures of *Drosophila* in water and in cane sugar at temperatures from 28° to 9°C., showing a temperature coefficient for the duration of life of about the order of magnitude of that of chemical reactions, namely of about 2 for a difference of 10° C. The averages for duration of life were much lower than those found by Hyde and Lutz because, of course, of the inadequate food. At 19° C. the culture in water had an average longevity of 4.1 days and those in 1 per cent. cane sugar of 12.3 days. In 1917 (65) the experiments were repeated, using sterile flies on 2 per cent. glucose agar which was found to be a more adequate food. Similar results were obtained in respect of the temperature coefficient for the duration of the larval and pupal stages. It was found that the ratios of the duration of the three different stages remained approximately constant for the different temperatures. The average durations of the life of the imago here given are more nearly of the order of those found by previous workers. 228 flies

at 30° lived an average of 13.6 days; 70 flies at 25°, 28.5 days; and 49 flies at 20°, 40.2 days. Later in 1917 Loeb and Northrop (66) published another paper in which they give 92.4 days as the average duration of life of 143 flies at 15°, and 120.5 days for 105 flies at 10° C., together with the frequency distributions from which the averages were obtained. They also studied the effect of different food mixtures, and the longevity of the two sexes separately, finding that isolated males live a little longer than isolated females, or than the males when mixed with females.

In 1917 Northrop (76) reported the results of some experiments undertaken to determine the effect upon the duration of life of the imago of prolonging the life of the larva by inadequate feeding (omitting yeast for different lengths of time). In this way the embryonic periods were prolonged from 8 to 17 days, but the duration of life of the adult remained the same in every case, ranging between 10.5 and 11.9 days at 27.5° C., at which temperature the four experiments, involving 644 flies, were performed.

Northrop has lately (1925 and 1926) returned to the study of duration of life in aseptic cultures of *Drosophila*. After the aseptic cultures had been carried on in the dark for over 200 generations, he (78) tested the effects of various intensities of light upon duration of life, with the following results:

"1. The duration of the larval period is shortened slightly at intensities around 2,500 meter candles, but

becomes increasingly longer at higher intensities. The larvae are killed by continuous exposure to light of 7,000 to 10,000 meter candles.

"2. The pupae are killed at intensities greater than 5,000 meter candles.

"3. Above 1,000 meter candles the duration of the imago period is rapidly shortened.

"4. The duration of life of the imago at different intensities of illumination can be quite accurately predicted by assuming that the light produces an independent 'rate of aging' which is proportional to the intensity of the light.

"5. The result of short exposure of the imago shows that light does not merely accelerate the normal 'rate of aging' and also that the effect is only partially reversible.

"Diffuse daylight does not affect the upper temperature limit of growth."

Northrop next attacked (79) the question of whether there is any correlation between total duration of life and rate of energy transformation during life, as was originally suggested by Rubner (123) and supported by Pearl (87). Aseptic cultures of *Drosophila* were reared at various temperatures and under differing light conditions, and their total carbon dioxide production measured. The conclusion reached is that: "The total duration of life . . . is not determined by the time required to produce a limiting amount of CO_2." Only average figures are given for life duration, and the general lack of any detailed description of crucial elements in the experimental situation makes

the critical evaluation of the tabled results somewhat uncertain. The problem needs further study.

In his most recent contribution Northrop (80) compares the duration of life of the flies of the 230th generation of the aseptic culture with normal wild flies under several different environmental conditions. The general conclusion reached is that: "The larval period and the pupal-imago period were found to be nearly the same for both cultures under both favorable and unfavorable conditions. There is no evidence, therefore, to show that inbreeding, absence of light, or growth in the absence of bacteria for 230 generations has had any effect either on the duration of life or on the ability of the organism to resist unfavorable bacteria." It is to be noted, however, that none of Northrop's tabled average durations of life for either aseptic or normal control flies is much more than half the average longevity which we have been accustomed regularly and constantly to obtain for normal flies in hundreds of experiments during the past seven years in this laboratory. Either he is dealing uniformly with hereditarily short lived strains of *Drosophila,* or else all of his conditions of fly husbandry are such as not to permit the realization of physiologically normal life duration.

Bliss (7) has lately studied the time duration of the prepupal stage of development of *Drosophila,* in relation to temperature, and concludes that between 12° C. and 30° C. the rate of prepupal development, taken in three ranges 12°–16°, 16°–25°, and 25°–30°, can be fitted by

three separate applications of the Arrhenius equation relating the velocity of an irreversible chemical reaction to temperature.

Turning to other animals than the fly, the most extensive systematic experimental work has to do with rodents. Greenwood and Topley (35, 130) have given life tables for mice, under controlled experimental conditions, in connection with their studies of epidemiological problems. Robertson (121 and earlier papers there cited) and his co-worker Ray, have made extensive observations on the normal life duration of the mouse and its extension following the administration of tethelin.

Noyes (81) presented data on the normal life duration of a rotifer, *Proales decipiens,* from which Pearl and Doering (97) computed a life table for this form. Finesinger (26) has also studied experimentally the duration of life of another rotifer, *Lecane* (*Distyla*) *inermis.*

Philip and Nellie Rau (115–117) have published important data on the duration of life in saturniid moths which do not take food in the imaginal stage.

Vinokuroff (131) found the mean duration of life of *Musca domestica* to be 1.3 days when starved and without water and 1.8 days when starved, but given water.

Kopec (54) has published the results of extensive studies on the effect of intermittent starvation of the caterpillars of *Lymantria dispar.* The moths do not take food in the imaginal stage. " Starvation of caterpillars has no distinct effect on the duration of the imaginal stage." But *total* duration of life, from hatching to death is pro-

longed by the intermittent starvation because of the extension of larval life. This prolongation may amount to 25 to 30 per cent. relative to the controls.

Glaser (27, 28) has contributed some interesting and valuable experimental results regarding the effect of different food conditions upon the duration of life in several species of flies. He notes that in the total absence of food *Musca domestica* lives only from one to two days. The numbers dealt with in these particular starvation experiments were small (12 flies all told) and it is impossible to construct a life table on such a meager basis.

Roubaud (122) has studied experimentally the fecundity and longevity of the domestic fly (*Musca domestica*), but gives detailed numerical data only regarding fecundity. He found the maximum duration of life at 20°–25° C. to be 66 days.

Wollman (139) reared *Musca domestica* under aseptic conditions, with sterilized milk as food. He published figures on the duration of life of six flies, under these conditions, and the range was from 26 to 62 days.

Dönhoff (21) described a method by which a life table for the honey bee might be constructed, but gives no precise numerical results of his observations.

In 1920 Arendsen Hein (1) published a few observations on duration of life in the meal-worm *Tenebrio molitor*. Thirty-two male beetles lived an average of 60 days, with a range from 39 to 113, and 32 females averaged 111 days, with a range from 89 to 132 days.

A careful series of observations, collected by a sound

experimental technique on the duration of life of *Hydra* has been furnished by Hase (45). The range of longevity was from 4 to 167 days.

Goetsch (30–32) has also studied experimentally the problem of life duration in *Hydra,* but owing to the complications introduced by alternative sexual and asexual modes of reproduction in this form his results have little direct bearing upon the problems to be discussed in this book.

Harms (44) in 1912 published data on *Hydroides pectinata,* from which death rates for this form may be calculated for a portion of the whole life span, but they are inadequate for the computation of a life table.

Some general observations on the duration of life of various slugs of the genera *Arion* and *Limax* have been reported by Künkel (57) but his experimental work with these forms was not particularly concerned with the problem of longevity.

Blunck (8) has made interesting observations on the duration of life of *Dytiscus,* finding that on the average the adults live from one to two years, and exceptionally to as long as two and one half or three years. The females are somewhat longer-lived than the males.

Labitte (58) collected observations during many years on the normal duration of life of beetles of various species. The average results are given in the following table.

It will be noted from this table that generally the mean duration of life was greater in the females than in the males.

Mean duration of life (in days) of various beetles.
(From Labitte 58)

	Males	*Females*
Procrustes	374.10	338.20
Carabus	323.20	385.71
Necrophorus	232.33	291.50
Dytiscus	853.66	740.00
Hydrophilus	164.66	374.00
Melolontha vulgaris	19.20	26.81
Cetonia aurata	57.50	88.00
Lucanus cervus	19.16	31.72
Dorcus	327.00	375.33
Ateuchus	338.25	466.80
Sisyphus	198.40	266.50
Copris	496.55	623.44
Geotrupes	700.06	642.14
Oryctes	37.50	55.50
Blaps mortisaga	848.20	914.40
Blaps gigas *Blaps magica* *Blaps Edmondi*	700.00	727.66
Akis	854.40	951.42
Pimelia	669.08	714.18
Timarcha	135.00	181.66

Further discussion of the pertinent literature will be incorporated where necessary in the body of the text. The general impression left by a systematic review of the matter is that the experimental work on the problem of

life duration, with the exception of the studies of Loeb and Northrop, has been of a highly scattered sort and has made but little real impression on the problem. A good part of it has been undertaken with entirely other objects primarily in mind.

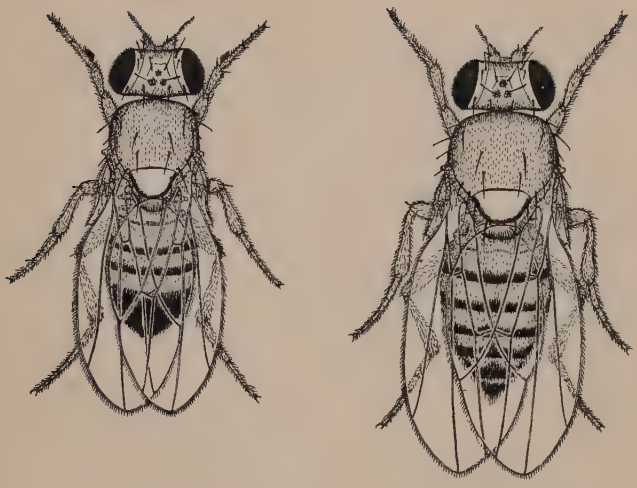


FIG. 1. Male and female of *Drosophila melanogaster*.

CHAPTER II

TECHNIQUE

WHAT is to follow in this book has to do mainly with various aspects of the biology of the fruit fly, *Drosophila*. It will help to an understanding of the experimental results to be presented if an account is first given of the methods by which the vital statistics of this animal are collected.

Drosophila melanogaster is a small fly, belonging to the sub-family *Drosophilinae* in the acalypterate division of the family *Muscidae* (129). Its appearance is shown in Fig. 1.

The life cycle of the fly is a short one, which makes it highly suitable for experimental work. The egg is laid by the female in the upper 1 mm. of the food substratum. About 2 to 3 days after the just-hatched female is placed in the container, larvae appear. The larva or maggot squirms about and feeds in the rich medium in which it finds itself for about 3 to 4 days and then forms a pupa. From the pupa the winged imago or adult form emerges in about 4 or 5 days. The female generally begins to lay eggs within the first 48 hours after she is hatched. So then we have about 9 to 11 days as the minimum time duration of

a generation. The whole cycle from egg to egg, at ordinary room temperature, falls within this 11-day period. Studies have been made by various workers, notably Loeb and Northrop (66), on the influence of various external factors upon the duration of the developmental stages in the life cycle.

In the laboratory the flies are cultivated for experimental work in glass bottles. The stocks are carried in half-pint bottles. Fig. 2 shows the appearance of such a bottle.

In the bottom of the bottle is the agar food material. The photograph shows how the larvae tunnel and mine this material next to the wall of the bottle. On the sides of the bottle are seen the dark pupae. The stopper has a hole bored through it in which fits tightly a piece of brass tubing 17 mm. in diameter, and 25 mm. long to the bottom of which is soldered a piece of screening, made of fine brass wire, having meshes so small that a fly cannot get through them. This ensures proper ventilation of the interior of the bottle. At the beginning of our work with *Drosophila* we followed the usual practice in American laboratories of stoppering with a wad of cotton. But plainly such a stopper permits very little if any circulation of air. An experimental test (103) showed that there was a significant increase, amounting to about 10 per cent., in the mean duration of life of flies kept in well ventilated bottles, with the openings covered with fine meshed silk bolting cloth, as compared with bottles stoppered in the ordinary way with tight cotton plugs. Following this ex-

FIG. 2. A half-pint milk bottle, as used for standard laboratory cultivation of *Drosophila*.

perimental test the perforated and screened cork stopper described above was devised and has since been used in our work.

Drosophila is generally cultivated upon the following medium, which originated in Morgan's laboratory.

H_2O	500 c.c.
Agar-agar	10 gr.
Banana pulp	500 gr.

Boil agar until dissolved — about 10 minutes. Mash bananas and add to agar and water and boil for five minutes. Bananas must be ripe but not rotten. Pour into bottles for use. Allow to cool and sprinkle lightly with yeast.

Some slight variations in the preparation of this medium, such as neutralization to litmus before adding yeast, allowing the flies themselves to seed the medium with yeast, etc., have been practiced by various workers, but the essential character of the medium as cooked banana pulp solidified with agar has been the usual laboratory procedure in working with this fly. In their most recent publication on the biology of *Drosophila,* Morgan, Bridges and Sturtevant (72) have the following to say regarding food (loc. cit., p. 6–7):

" For laboratory purposes, bananas furnish by far the most desirable culture medium, but the fly can also be reared on other fermenting fruits. In our culture bananas are used, but whether there is something supplied by the banana to the flies that is advantageous, or whether the banana is the best medium for the growth of yeast, that

appears to be the chief element of the food for both flies and larvae, is not known. It is not improbable that the relative absence of moulds such as mucor, etc., in the acid banana cultures also plays a rôle in the results."

The work of Guyénot (36–43), Loeb (64), and Northrop (77), and Baumberger (3), has shown that any notion that fruit in any form is in any way necessary for any biological process in *Drosophila* is not true. Baumberger found that the flies "can develop normally on yeast nucleoprotein, sugars, and salts."

Several years work with this banana medium convinced us that it had many undesirable features, especially when exact quantitative work was to be done. In particular its composition is in no wise constant. Bananas vary greatly in different seasons of the year in their biological qualities at least, and presumably in their chemical composition. Consequently we (93, 96, 107) carried out an investigation extending over the greater part of two years to devise a more suitable food medium, of standard, known, and constant composition.

The medium finally adopted had the following composition:

Synthetic, S–101

Solution A.	Cane sugar	500	gms.
Rochelle salt	$(KNaC_4H_4O_64H_2O)$	50	"
	$(NH_4)_2SO_4$	12	"
Epsom salt	$(MgSO_47H_2O)$	3	"
	$CaCl_2$	1.5	"
	H_2O to make 3000 c.c. of solution		

Solution B. Agar-agar	135	gms.
Tartaric acid ($C_4H_6O_6$) ..	30	"
KH_2PO_4	6	"

H_2O to make 3000 c.c. of solution

Melt the agar thoroughly in the water *alone,* with heat, then add the salts to complete Solution B., and for the medium to be used in the fly bottles, mix equal parts of solutions A and B. For some kinds of work it has proved desirable to have the final food a little less stiff, in which case a small amount of agar is used, without changing the composition otherwise.

This medium has a pH when freshly made, cooled, and the agar set, of approximately 3.7. As the flies live upon it the pH falls to a value of 3.0, or in some cases even lower.

This new medium has been tested in a great variety of ways in the laboratory. It has proven so satisfactory that all our *Drosophila* stocks are now carried on it, as a routine. On account of its high acidity there is practically never any contamination of the cultures by troublesome bacteria. In particular *Bacillus subtilis,* which can make a great deal of trouble on the standard banana medium, never gets a foothold on this medium S–101. Some moulds will grow on it, but the trouble from this source in routine *Drosophila* work practically disappears by the use of this medium.

Experimental tests, the details of which are given in (96), show conclusively that, aside from the practical

advantages in respect of fly husbandry noted above, this medium S–101 presents an environment much nearer the

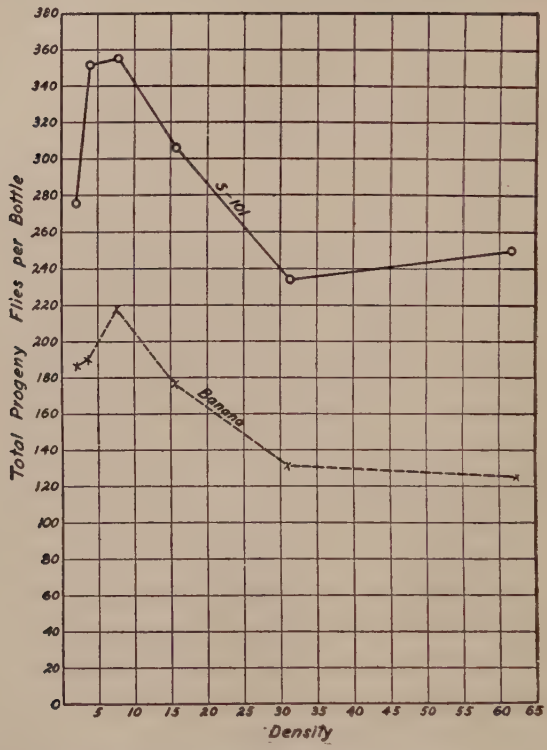


FIG. 3. The average absolute number of progeny flies per bottle produced in the S–101 series (solid line) and the banana series (broken line). From (96).

biological optimum than does the banana medium. This is shown for fertility in Figs. 3 and 4.

The parent productivity, as measured by imagoes produced per female per day, is higher on the S–101 medium at all densities than on the standard banana medium. The

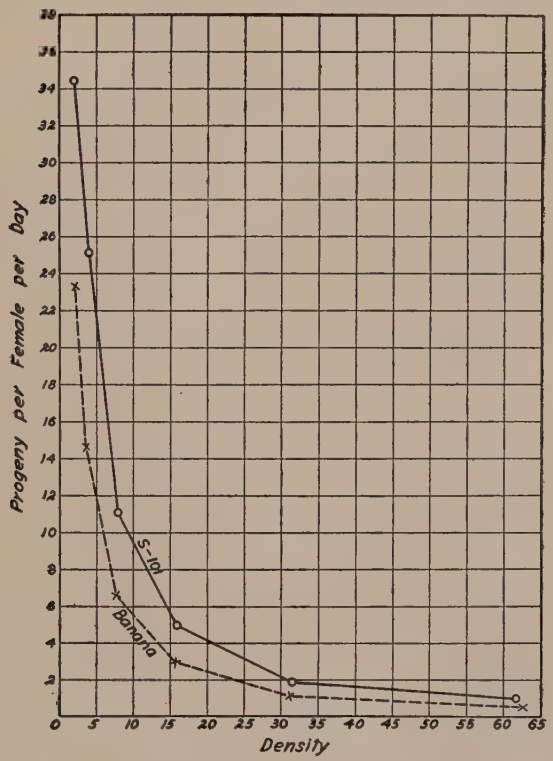


FIG. 4. Progeny per female per day on the synthetic medium S–101 (solid line) and on the standard banana medium (broken line). From (96).

relative amount of this excess is shown by the following figures.

Percentage Increase in Fertility (Progeny Produced per Female per Day) on the Synthetic Medium S–101, as Compared with Standard Banana Medium

Initial Density	Percentage increase of S–101 over banana
2	47.7
4	71.8
8	68.3
16	68.0
32	62.9
64	98.0

There can be no doubt that the production of progeny, however measured, is much higher on the synthetic medium than on the standard banana.

The same superiority of the synthetic medium is also apparent in respect of duration of life. In some preliminary experiments on the point, over a period of 8 days, only 8.73 per cent died out of 252 flies exposed to risk of dying in the S–101 bottles, while in the same time 26.98 per cent. of 252 flies exposed to risk in banana bottles died. The mortality was relatively three times as great on the banana medium as on the synthetic. Subsequent life table experiments on a more extended scale have confirmed the finding that a higher average duration of life is attained on the S–101 medium than on the banana.

The routine experiments on duration of life are carried out in specially moulded one-ounce bottles, with a neck

fitted to receive a perforated cork like that described above but of smaller size. In each bottle is placed 5 c.c. of food medium. Flies are taken immediately after emergence from the pupal stage as imago, the time of this emergence being taken always as zero age.

In order to make up the population of each experimental bottle accurately the just-emerged flies are lightly etherized, and with the aid of a binocular microscope, the sex of each fly is observed. While anaesthetized the desired number of flies of each sex is then counted into the bottle. In order to determine whether anaesthetizing by ether itself influenced duration of life, a series of experiments was made (101). In these experiments the ether dose used was constant for all the flies. The group to be etherized was shaken into a clean half pint bottle; 5 c.c. of ether was poured on a piece of absorbent cotton fastened to the under side of a cork stopper; the bottle with the flies was stoppered tightly with the cork and left for two minutes. Then the flies were turned out on a tile and sexed and counted (since that operation corresponds in extent of handling to what we need to do in making up matings, etc.), then emptied into a vial with fresh food, where they recovered from the ether in about half an hour. For each successive group of flies a fresh bottle and fresh cotton for the ether were of course used.

Seven series of experiments were conducted, differing in respect of the number of times the flies were etherized, and in their age at the time of etherization.

The seven series were as follows:

A. Etherized once when one hour of age.

B. Etherized once when twelve hours of age.

C. Etherized once when thirty-six hours of age.

D. Etherized once when three and a half days of age.

E. Etherized twice when seven, and fourteen days of age, respectively.

F. Etherized three times when seven, fourteen, and twenty-one days of age, respectively.

G. Etherized four times when seven, fourteen, twenty-one and twenty-eight days of age, respectively.

The results of these experiments are shown in Table 2.

TABLE II

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF ETHERIZED AND NORMAL DROSOPHILA

Treatment	Number of flies	Mean (in days)	Standard Deviation (in days)	Coefficient of Variation
Etherized when 1 hour old..	428	50.82 ± .38	11.76 ± .27	23.14 ± .56
Etherized when 12 hours old	443	50.20 ± .39	12.25 ± .28	24.41 ± .59
Etherized when 36 hours old	411	53.36 ± .46	13.91 ± .33	26.06 ± .65
Etherized when 3½ days old.	432	54.50 ± .40	12.36 ± .28	22.68 ± .55
Etherized when 7 and 14 days old.....	445	51.43 ± .40	12.50 ± .28	24.31 ± .58
Etherized when 7, 14, and 21 days old.....	413	51.72 ± .50	15.01 ± .35	29.03 ± .74
Etherized when 7, 14, 21, and 28 days old.....	420	49.26 ± .47	14.42 ± .34	29.26 ± .74
Controls (taken out of mating bottles when 1 hour old).....	478	51.11 ± .42	13.75 ± .30	26.90 ± .63

From these experiments it was concluded that no sensible error will be introduced into duration of life experiments on *Drosophila* as a result of completely anaesthetizing the flies with ether at least up to as many as four times in the course of their lives.

Since all the one-ounce bottles in a life duration experiment are started with flies which have just emerged it follows that all the living flies in any such bottle at any time are of the same age. Each day after the start of an experiment the bottles are examined, dead flies removed, sexed, and recorded as to duration of life. Every second day, as a routine procedure, the flies are transferred to a fresh bottle with new food. In the earlier experiments different plans were adopted as to the frequency of transfer to new food, though always the same procedure was followed in any particular experiment. In recent years, however, we have followed the plan of changing every other day regularly. In making these transfers the flies are not etherized.

All of the experiments on duration of life are carried out in electric incubators operating at 25° C. The bottles are only taken out of the incubators for the short time necessary for the daily examination and removal of dead flies.

In experiments on reproduction, fertility and population growth the fundamental elements of the technique described above are followed as a routine. But inasmuch as such experiments involve frequent, often daily, census counts of living flies it was necessary to devise a method

by which this might be done without etherizing the flies. The plan adopted was to use a "counting tube" (101) of the form shown in Fig. 5.

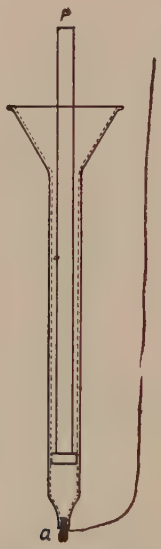


FIG. 5. Diagram showing construction of *Drosophila* counting tube. The aperture at *a* is just large enough to allow one fly to pass through at a time. The essential dimensions are as follows: Length over all 25 cm., diameter of main tube 2 cm., diameter of funnel mouth 6 cm.

When it is desired to count a definite number of flies the small aperture *a* is temporarily plugged with a bit of cotton wool, the plunger *P* is removed from the tube and flies are shaken into the counting tube by inverting the open bottle containing them over the funnel mouth of the counting tube. Then the plunger is inserted and gently moved forward to concentrate the flies in the lower end of

the counting tube. Then the counting tube with enough cotton around it to close up the mouth of the bottle is inserted into the bottle into which it is desired to place the counted flies and the plug removed from the aperture *a*. Then as the flies come out of the tube, one by one, through the aperture *a*, they are counted as they pass this point, with the aid of a tally register, such as is used by doorkeepers at theaters, etc. The plunger is gently moved forward as necessary to keep up an even flow of flies through the mouth of the tube.

While the great bulk of the experiments to be described have been carried out with the normal wild-type *Drosophila melanogaster*, considerable use has also been made of one of its mutations, first discovered in Morgan's laboratory and described by him (69), and later in greater detail by Bridges and Morgan (10). The characteristic appearance of the wings in this mutant form is shown in Fig. 6.

Deviations from the experimental technique above described, and special additions to it for particular ends will be described in the text as occasion demands.

Throughout this book it will be assumed that the reader is familiar, at least in a general way, with the ordinary biometric and actuarial mathematical procedures and terminology. Elementary text-book treatments of these matters will be found in the following references: (140), (88), (48), and (29). Any difficulties the reader may have in following the mathematical technique here used will be removed by consulting these books.

So far the discussion in this chapter has been of details of technique. I wish finally to say a few words about biological experimentation in general. It is beyond question the most difficult of all forms of scientific experimentation, as has been noted by many persons, not the least of whom was one of the most brilliant biological experimenters ever known, Claude Bernard (5). In order to get correct results from experiments on living organisms it is essential among other requirements, to have extensive prior knowledge of the peculiarities and idiosyncracies, both physiological and psychological (behavioristic) of the particular organism used as subject. Such knowledge can only be acquired by living with that organism for a long time and studying it from many points of view. All of this is generally speaking, not required in physical and chemical experimentation, or, at least, in nothing like the same degree. Hydrochloric acid for example, remains hydrochloric acid every time it is tried.

Anyone who knows any particular animal or plant thoroughly is usually impressed, I think, in reading the literature of biology, that a great deal of the published experimental work on that form is really of dubious validity, because of the generally casual manner in which it was undertaken and prosecuted. Firmly established basic backgrounds in the genetics, behavior, physiology, and practical husbandry of the form are too frequently absent from the crucial experimental picture.

Of all methodological procedures in biology the *experimentum crucis* is the most dangerous. A great deal is heard

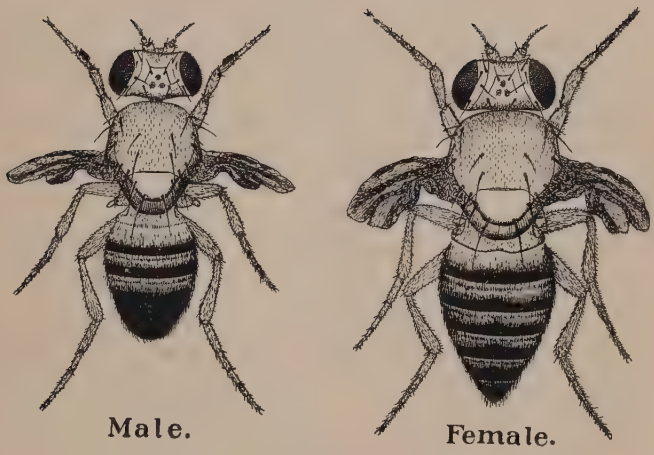


FIG. 6. Male and female of vestigial mutant of *Drosophila melanogaster*.

to the effect that the crucial experiment is the only thing that really counts. All other types of biological methodology are contemptuously characterized as " vague " or worse. But nothing emerges more clearly from the history of biological thought than that, almost without exception, the crucial experiments which have been most loudly hailed at the time they were made, as forever settling the problem under discussion, have been subsequently found to have led to quite erroneous conclusions. I shall take the space to present but a single example. Up to the time when Jennings began his work on the subject virtually all the investigation of the tropisms of unicellular organisms had been done by the " crucial experiment " technique, and it was held that the Loeb theory of tropisms had thereby been firmly established forever. But the *real* point, the essential element, in the behavior of these creatures had been *completely* missed. And as any intelligent person could subsequently see, the *experimentum crucis* type of procedure is nearly, if not quite, the worst conceivable method which could have been used in an attempt to discover the real point, namely the " motor reflex " which is the fundamental mechanism of ciliate behavior. A safe working rule is to be extremely suspicious of any conclusions that are said to be " demonstrated " by crucial experiments alone.

Something of the point of view under discussion may be gained if one considers what the results would be likely to be if a human being were subjected to the sort of treatment which animals are commonly given in the

laboratory. Would his reactions under such circumstances be normal, or of a sort likely to lead to true insight as to his physiology and psychology? A favorite method of studying the effect of alcohol upon animals, for example, has been to insert a catheter through the mouth to the stomach and pour a measured dose of the alcohol down the tube. Fancy a super-giant biologist from Mars studying by this technique the reaction of human beings to *Chateau Haut-Brion!*

The general viewpoint regarding biological experimentation here discussed has been extensively developed, from another angle, by Krohn (56). He enunciates the following general principle as the proper guiding one in biological experimentation: "If the quantitative relations of the effects of the various forms of expression of a physiological factor are to be investigated, the other remaining physiological factors necessary for the existence of the organism must be arranged according to their optimum values in respect of every form of expression of the particular physiological factor under consideration, and the maximum effects thus obtained compared with one another."

Years of experience have led to two conclusions. The first is that it takes months or even years of careful observational work to get ready to do a really significant biological experiment. This has been particularly true in the experimental biostatistical work with flies. In the aggregate many months have been spent in studying matters of fly husbandry, for the sole purpose of learning how to

set up definitive experiments which would have in their final results some degree at least of consonance with significant reality.

The second conviction arising from experience is that the much greater variability and lack of precise repetitive uniformity which is commonly supposed necessarily to inhere in biological phenomena, as contrasted with inorganic physical and chemical phenomena, is in no small measure the simple result of general sloppiness and lack of specific, pertinent backgrounds of general knowledge in the conducting of biological experiments. When the biologist exercises something approaching the same precision and infinitely painstaking care, over *all* the most trivial details of a biological experiment that the physicist does over his, the results tend to take on a degree of precision and uniformity not so far short of that usual in the older science, as we are accustomed to expect. The biologist has something to learn from the biometrician besides methods of computing, and from the physicist besides the quantum theory.

CHAPTER III

LIFE TABLES

A LIFE table is a mathematical description of the age distribution of death in a group of organisms. We may well begin our study of group biology with its consideration. For it connects death rates, which are biological attributes of the group, with the age of the organisms in the group, and age is a highly important biological attribute of the individual.

To speak of the death rate of an individual has no meaning. During all the time that the individual is living there is one person exposed to risk of dying, who does not die, and therefore exhibits a death rate of zero. At the instant he does die there is one person exposed to risk of dying who expires at that moment, and in so doing exhibits a death rate of one. But for this unfortunate individual the death is the biologically significant thing, not the rate. The case is quite different for the group. Within any defined time interval there are n component units of the group exposed to risk of dying, and m die, leading to a death rate which defines, for that particular time, a vital phenomenon of the group, which has been called the force of mortality. The life table is a mathematical device for

describing precisely the connection of this vital phenomenon with the age of the individual units composing the group.

When our studies in the field of experimental biostatistics were started there existed a complete life table for but one form of life, man. There were no data available which permitted the construction of a life table for any other organism. Since then life tables have been published, in whole or in part, for *Drosophila* and several of its mutants (86, 99, 104), for the rotifer *Proales decipiens* (97), for the moth *Telea polyphemus* (104), and by Greenwood and Topley (35) for the mouse.

The definitive life tables for *Drosophila melanogaster* (normal wild type) and its mutant vestigial are given *in extenso* in Appendix I of this book, together with details as to their computation. They were originally published in (104).

The general form of the survivorship curves is shown in Fig. 7.

On the whole the graduations are reasonable. All that we were concerned to do was to describe satisfactorily the general sweep of the observations. The actuary, by the use of what amounts to a curve of many constants, fits some of the minor fluctuations in the observations as closely as possible, and unquestionably preserves in his final results a good many irregularities which are really only sampling errors. There was no such desire in this work. The curves for vestigial flies are close fits up to 31 days in the females and 49 days in the males. The upper tails of both these

curves for vestigials are bad fits, underestimating the observations in the females and overestimating in the males.

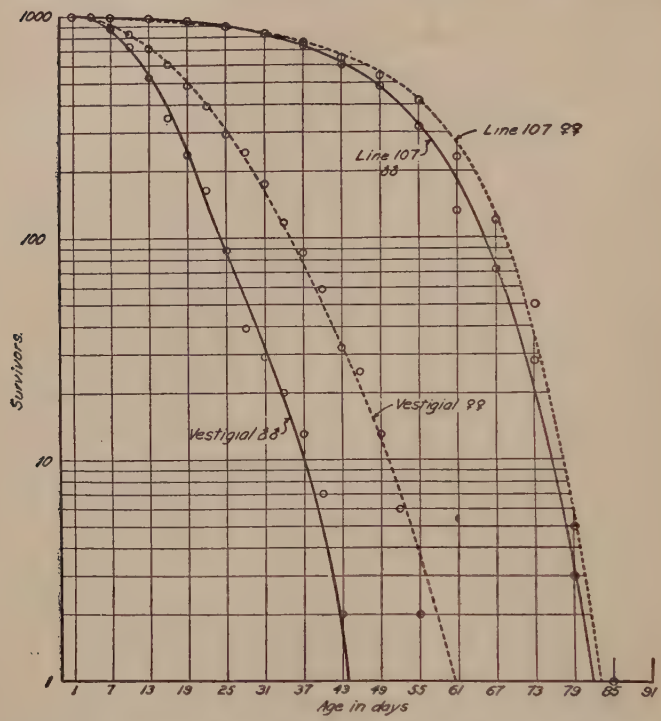


FIG. 7. Diagram showing the observed and graduated l_x points for (a) line 107 wild type, and (b) vestigial flies. The small circles are the observations from Tables 17–20 in Appendix I, and the smooth lines the fitted curves from the equations there given.

To take care of these end observations would require additional constants in the equations. But for all purposes to

which fly life tables are ever likely to be put the present graduations will probably be adequate.

The life tables show a number of points of general interest. In the first place there is an evident general resemblance in form of the normal wild type curves to that portion of human life curves which runs from about the age of 12 years on to the end of the life span. This resemblance will be more particularly discussed farther on.

In the second place these tables show the same general relation between the sexes in respect of mortality that human life tables do. Except in the early portion of the imaginal life span (and also at the extreme upper end in the case of wild type flies) the female flies exhibit smaller death rates at ages than do male flies. The expectation of life of a wild type male fly at emergence is 45.8 days, while that of a female fly is 48.0 days. In the vestigials the expectation of life of a male at emergence as an imago is 14.1 days, and of a female 19.8 days.

It seems to be a rather general phenomenon, among groups of organisms in which there is sexual dimorphism, for males to be shorter-lived on the average than females, but statistically adequate quantitative information about duration of life is so meager that any such generalization would be premature at the present time. Such cases as those of *Dinophilus apatris* and various rotifers discussed by Korschelt (55), in which the male is dwarfed and obviously deficiently organized, and at the same time is short-lived, as compared with the female, are perhaps to

be regarded as extreme illustrations of the dependence of duration of life upon bodily organization or pattern, a point which will be more fully discussed in later chapters. But these cases are not entirely probative evidence in support of a hypothesis that the organization or pattern differences implicate in normal sexual dimorphism of the kind and degree seen in man, for example, have generally as one of their normal expressions a shorter average duration of life in the male. Korschelt apparently inclines to the opinion that such a relationship is general, but makes the same point as is emphasized above, that the data available are insufficient to settle the question. It is of some interest to note that Blunck (8) finds the average duration of life of female beetles (*Dytiscus*) greater than that of males, and also that Labitte's (58) observations on the duration of life in beetles generally show the same thing.

The form of the life curve for vestigial flies is distinctly different from that for the wild type flies. The mortality of vestigials is characterized by a long period of slowly rising death rate values in middle life (in the males forming even a slight dip convex to the base). This phenomenon gives the survivorship curves for these flies their peculiarly flattened appearance in the middle portion of their course.

It will doubtless have occurred to the reader that the expectations of life of *Drosophila* at emergence stated above, are of the same general order of magnitude *in days* as the corresponding expectation of life of man at birth,

in years. This suggests that it will be desirable to superimpose the *Drosophila* and human life curves for purposes of more exact comparison. And in general the study of comparative mortality demands that some method be devised for overcoming the difficulty created by the fact that different animals have different life spans, in terms of absolute time units.

This fact offers no insuperable difficulty to the comparative study of the laws of mortality. What is needed is to superimpose the two curves so that at least two biologically equivalent points coincide. The best two points would be the beginning and the end of the life span. But in the case of *Drosophila* the life tables start with the beginning of adult or imaginal life only. The larval and pupal durations are omitted.

The problem then is to determine what part of the human life cycle is biologically equivalent to the emergence of the imago from the pupa case in *Drosophila*. There are many ways in which this question could be argued, differing in respect of the biological functions upon which emphasis was to be placed in determining equivalence. Owing to the fundamental differences in the embryonic development and physiology of a mammal and an insect with complete metamorphosis, any attempt to base age equivalence upon physiological characters would be likely to yield a different result for each different character taken. The logical procedure in the premises would seem to be to adhere rigidly to the field of discourse. It is desired in the present case to determine equivalent ages in respect of

the force of mortality. We can locate the starting point of the curves on this basis by putting the human and *Drosophila* survivorship curves together at the age for each organism *where the instantaneous death rate q_x is a minimum.* In the case of *Drosophila,* I think we are safe in concluding that this point is at or very near the beginning of imaginal life. We shall accordingly take *Drosophila* age 1 day as this point. The life tables show that certainly after this time q_x never again has so low a value. Indeed the fundamental law of mortality in *Drosophila* imagoes was stated (99) in this way: " the instantaneous death rate increases with age as a modified logarithmic function of x."

The definitive edition of Glover's (29) United States Life Tables gives (p. 68) for white males in the original registration states the following values for q_x: for age 11–12, 2.28, and for age 12–13, 2.29. We may, therefore, with sufficient accuracy take exactly 12 years as the minimum point, particularly as the survivorship figures we shall have to use are tabled as of the beginning of the age interval.

For the other end of the life span we may conveniently take the age at which there is left but one survivor out of 1,000 starting at age 1 day for *Drosophila* and age 12 years for white males.

On this basis the survivorship distributions from the *Drosophila* life tables are given in terms of relative ages (centiles of the imaginal life span) in Table 21 of Appendix I. The corresponding figures for human white males, computed from Glover's table, are given in (86).

In Fig. 8 these centile distributions for *Drosophila* are compared with similar data from (a) human life tables

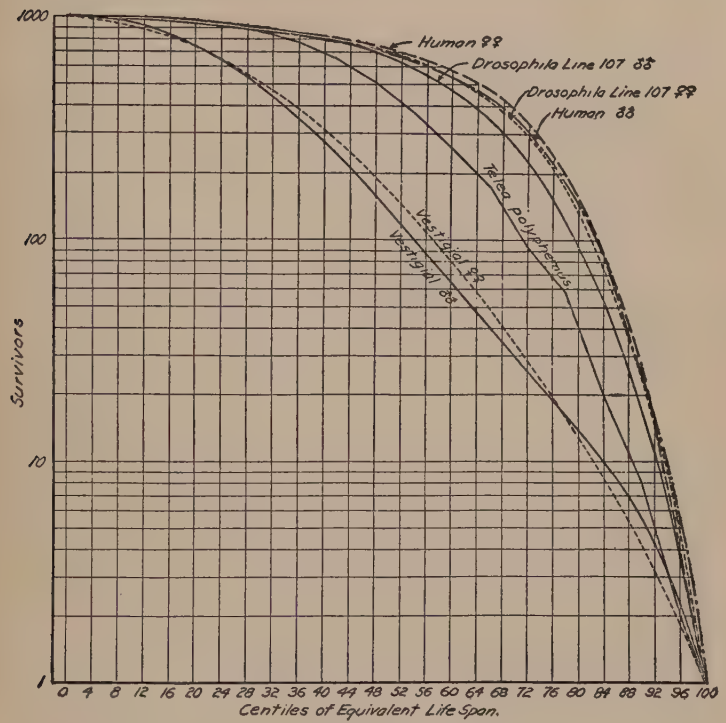


FIG. 8. Comparing the survivorship distributions of (a) *Drosophila* line 107 males; (b) *Drosophila* line 107 females; (c) *Drosophila* vestigial males; (d) *Drosophila* females; (e) human males; (f) human females; (g) *Telea polyphemus,* both sexes together, over the equivalent life spans.

(Glover (29)), and (b) the saturniid moth *Telea polyphemus,* on the basis of our calculations of an

ungraduated life table from data as to the duration of life of this form in the adult stage given by Rau (117).

From these life table curves several results of interest emerge. In the first place it is evident that the distribution of mortality in the different parts of the biologically equivalent life span is substantially identical quantitatively in an inbred strain of *Drosophila* (line 107) and in human beings of the present time. That is to say, if we take biological age as the base line, mortality is distributed along that base line in quantitatively the same manner in man and a particular inbred strain of wild type *Drosophila*. This does not, of course, in the least warrant the assertion that the forces determining rates of mortality in the two cases are identical. In detail they obviously are not. So far as is known, for example, the tubercle bacillus is not pathogenic to *Drosophila* while it is to man. The facts only mean that different in their qualitative details as are the lethal forces which attack the two organisms, man and a certain kind of *Drosophila,* they are alike in their quantitative relations to biological age. But this fact, which is after all a sufficiently remarkable one, suggests further that perhaps some underlying attribute of the organism, of which age is one expression, is vastly more important in determining when the individual shall die than are all the environmental forces taken together, within, of course, the environmental range compatible with the existence of living things at all. This is a point to which we shall revert farther on.

In another kind of *Drosophila,* differing so far as is known from the kind mentioned in the previous paragraph only in respect of one single second chromosome gene (and its somatic expression), the distribution of mortality in respect to biological age is widely different quantitatively from that found either in man or in wild type *Drosophila,* even in spite of the fact that both kinds of *Drosophila* spent their entire lives in physically and statistically identical environments, so far at least as concerns temperature, optimum population density and housing, food, season and climate. This fact seems quite as significant as the identity established in the preceding paragraph. It shows that a unit change in the genetic constitution of an organism may not only be associated with a marked alteration of the absolute length of the life span, but also with a profound alteration of the form of the life curve.

Wild type *Drosophila* and vestigial are plainly approximating two quite distinct theoretically possible forms of life curves. One of these types, which may be called the rectangular, would in the limit show all the individuals starting at birth together, living to the same age and then all dying together at the same time. The life table death rates at ages, q_x, would equal zero up to this "day of judgment," and then would on that day take the extreme value of 1,000 (on a per thousand base). The closest approximation yet seen in living nature to the theoretical limit is in *Proales,* as set forth by Pearl and Doering (97); the next closest approximation yet described is that

of *Drosophila* (line 107) and present day human beings, as shown in Fig. 8 above.

The other theoretical type of life curve which concerns the present discussion may be called the diagonal. This, in the limit, would be a case where the instantaneous death rate q_x would be constant at all ages from the start at birth to the demise of the last survivor. Plotted on arithlog paper the survivorship curve would be a straight diagonal line. The closest approach yet found in living nature to this theoretical type of life curve is that given by vestigial *Drosophila*, as shown in Fig. 8. All other life curves yet known fall between the rectangular and diagonal types.

There is a third type theoretically possible, but not actually realized in experience as yet. This is the case in which q_x has very large values in early ages, and thereafter nearly constant values until the last survivor is reached. This would mean a survivorship line which dropped sharply to a low level in the earliest ages and then ran along a nearly horizontal course to the end of the life span of the last survivor. This would be the life curve of a heavy selective mortality in early life. It is difficult to see how it could occur in a population genetically homogeneous in respect of factors influencing duration of life. But theoretically it could readily occur in a population genetically mixed relative to these factors.

One of the most important results of the definitive fly tables is that they suggest a multitude of interesting problems regarding the biology of death and of life duration.

We have worked upon many of these problems during the last eight years, but limitations of time and space will permit the discussion here of only a fraction of our results. The first query which suggests itself from the examination of the life tables which have been presented, is as to the extent to which the form of the life curve can be altered by environmental influences. Let us therefore examine the evidence on this point.

CHAPTER IV

DENSITY OF POPULATION AND LIFE DURATION

In the course of the work with *Drosophila* the effects of various environmental influences upon mortality have been studied. The character and amount of the food available to the flies influence their mortality rates to a certain extent, but unless the food relations are grossly outside the range of physiological normality the effects produced are not particularly startling. By improved ventilation of the bottles the mean duration of life was increased about 10 per cent (103). The effect of repeated etherization has already been discussed in an earlier chapter, and shown to be insignificant. Extensive experiments were tried (103) to determine what effect was produced upon the duration of life of flies by feeding embryonic materials, both from fly larvae and from chick embryos, to the amount of 2 per cent of the total food material. The general result of these experiments was negative. Certainly there was no prolongation of life.

Early in the work on experimental vital statistics it was found that there was one environmental factor which exerted a marked and systematic influence on duration of

life. This factor is the density of population in the bottles. In the first instance a general statistical analysis of our data (102) showed that density was influencing the results. Then systematic experiments on the matter were undertaken.

The first series of such experiments involved 13,000 flies, normal wild-type *Drosophila* belonging to an inbred strain called Line 107, of which the genetic history will be given in the next chapter. The food used was banana-agar, and the experiments were all conducted at 25° C. The general technique was that which has been described as standard in Chapter II.

The 13,000 flies in the experiment were divided among 530 one ounce vials, in 20 different densities, running as follows: 2, 4, 6, 8, 10, 12, 15, 20, 25, 35, 45, 55, 65, 75, 85, 95, 105, 125, 150, 200 flies per bottle.

In each bottle was put at the start an equal number of male and female flies, except that when the initial density was an odd number, one half of the bottles of that density had one more female than there were males, while the other half of the bottles in that series had each one more male than there were females. There were then in the whole lot at the start 6,500 males and 6,500 females.

Since the size of all the bottles was the same (1 oz.) and the amount of banana-agar put in each bottle was always the same (5 c.c.), density of population may most simply be indicated as the number of flies per bottle, and this usage will be followed throughout. The bottles were observed daily, and the deaths recorded as they occurred.

In such an experiment the density of population, as defined above, obviously must change with the history of the bottle. For example, suppose a bottle to start with 2 flies. At the end of say 14 days one of these flies dies. Up to that time the density of population in the bottle has been 2. After that time, and on till the death of the lone survivor, the density is 1. Now the first fly lived his entire life of 14 days exposed to a density of 2. But the second fly lived 14 days of its life exposed to a population density of 2, and, let us say, the remaining 18 days of its life exposed to a density of 1. So then the *mean density* (for which the symbol M.D. may be used) to which it was exposed over its entire life of 32 days was:

$$\begin{array}{r} 14 \times 2 = 28 \\ 18 \times 1 = 18 \\ \hline 32 \qquad\quad 46 \end{array}$$

Therefore, the mean density $= \frac{46}{32} = 1.44$.

In this way the mean density to which the fly was exposed during its whole life time was calculated for each of the 13,000 flies in the experiment. It was a long and laborious bit of computation.

Weighted means were then made of the mean densities for all flies started at the same initial densities. The basis of the weighting was the frequency of occurrence.

The examination of the results may best begin with the survivorship distributions. The detailed numerical values for these distributions are given in (98). A part of them

are shown graphically in Figs. 9 and 10. All of the lines are not shown in the drawings because to do so would con-

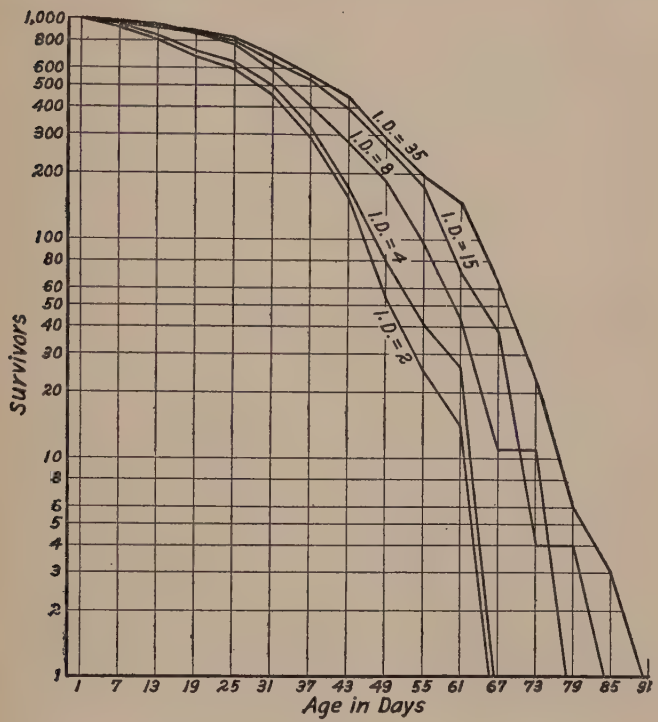


FIG. 9. Survivorship distributions for initial densities of 2, 4, 8, 15, and 35 flies per one ounce bottle.

fuse the picture by crowding. The lines which are omitted show nothing different in principle from those that are given.

It is at once apparent from these diagrams that density of population has a marked influence upon life duration. The highest line in each diagram is that for initial density

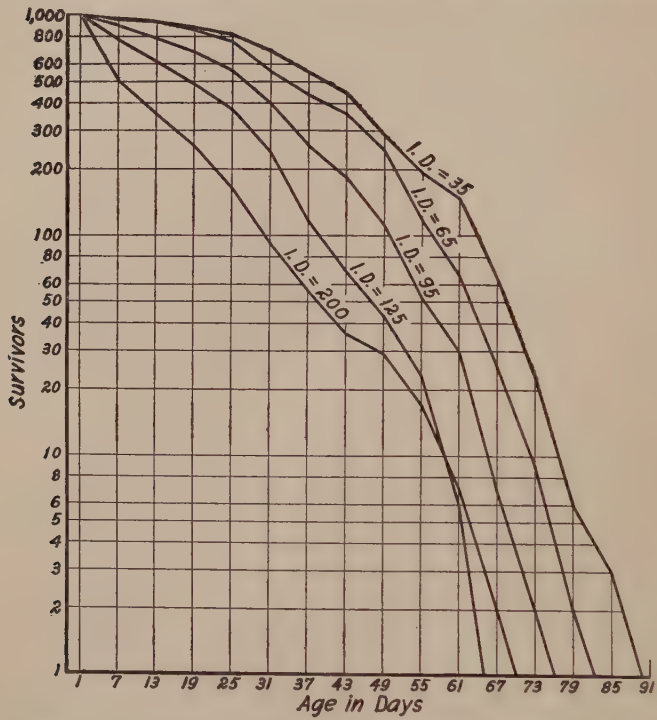


FIG. 10. Survivorship distributions for initial densities of 35, 65, 95, 125, and 200 flies per one ounce bottle.

35. This means that at about that density the flies, on the whole, survived longest. Much lower or higher initial densities were less favorable to survival under the condi-

tions of the experiments. The form of the survivorship distributions undergoes a gradual alteration on either side of the optimal density. This is particularly true as we go towards higher densities, until finally at density 200 the survivorship curve on arithlog paper approaches the straight diagonal line which indicates a constant death rate at ages.

The profound effect of density of population upon duration of life in these flies is also shown by the death rates at ages, which are recorded in detail in (98). For the six age groups (in days) 1–6, 13–18, 25–30, 37–42, 49–54, and 61–66, these death rates are shown graphically in Fig. 11.

The specific death rates in these experiments conformed in their behavior to the following principles, with, of course, fluctuations due to sampling errors. In the first place the death rates, at all densities up to about 100 flies per bottle, tend to increase with age, in the normal manner expected from the *Drosophila* life tables discussed in Chapter III, and from all the studies on the mortality of this fly. At densities beyond 100 to 125 flies per bottle there is a high death rate at the start (lowest ages), which tends to fall with advancing age up to roughly the middle of life, and thereafter to increase with advancing age.

At all ages the death rates start at a relatively high point in the lowest density (2 flies per bottle) and tend thereafter to *decline* with increasing density until densities of roughly 35 to 55 flies per bottle are reached. Thereafter, as density of population increases, the course taken by the specific death rates is different in the different age groups.

In the youngest age groups the death rates increase steadily with increasing density, after the optimal density is

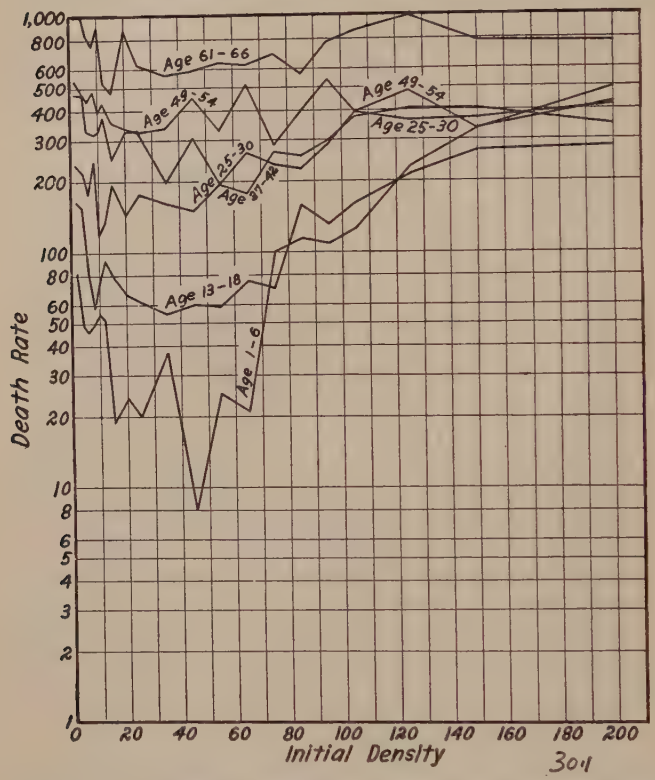


FIG. 11. Changes in specific death rates with advancing initial densities of population, for six age groups.

passed, right away to the highest densities tried in these experiments. But from the age group 25–30 days of age on

to the end of the life span there is virtually no increase in death rates associated with advancing densities of population, after a density of 100 flies per bottle is reached. In general, as is plainly shown in Fig. 11 the death rate curves, for constant age and changing density, tend to become flatter (that is to approach more nearly to straight horizontal lines) as age increases.

The facts which have been so far set forth regarding the influence of population density upon mortality seem to support the following conclusions.

1. The rate of mortality of *Drosophila* is profoundly influenced by density of population, that is, by the number of flies together occupying a limited universe in which volume of air, volume of food, and area of food surface are constant.

2. There is an optimal density of population for *Drosophila,* under the conditions of these experiments. It falls somewhere in the region of 35–55 flies per one ounce bottle containing 5 c.c. of food substrate. At densities of population above and below the optimum the specific rates are higher, at all ages, than they are at the optimum.

3. The deleterious effect of non-optimal densities of population is most pronounced at the beginning of imaginal life. In the first few days after the fly emerges from the pupa case it is extremely sensitive to influences consequent upon the size of the group of which it is one component member. As the fly grows older differences in either initial or mean density of population have associated with them relatively smaller differences in rate of mortality.

The nature of these changes in duration of life associated with different densities of population may be shown in a different way, which brings out clearly some additional facts, by calculating the ordinary biometric constants from the distributions of deaths in respect to age. These constants are exhibited in Table 3.

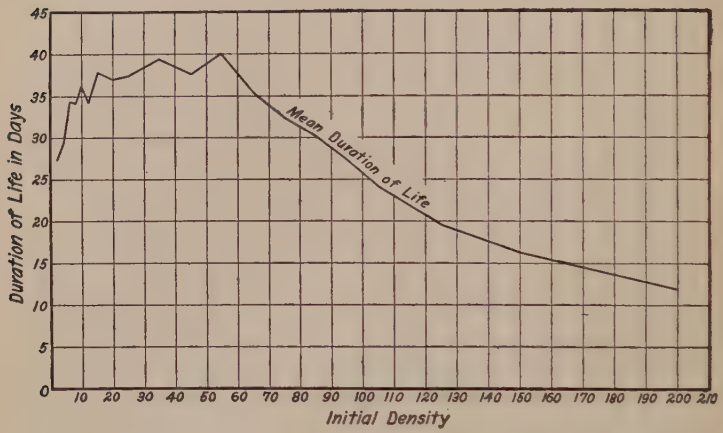


FIG. 12. Mean duration of life of wild type *Drosophila* at different densities of population.

The mean or average durations of life given in that table are shown graphically in Fig. 12.

Between initial densities of 2 and 15 flies per one ounce bottle the mean duration of life increases rapidly with increased density. Between densities of 15 and 55 flies per bottle there is a slow and gradual increase in mean duration of life. In fact it is not certain that this region of the

TABLE III

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF DROSOPHILA AT DIFFERENT DENSITIES OF POPULATION

Initial density	Weighted average mean density to which flies had been subjected up to time of death	Duration of life in days		
		Mean	Standard deviation	Coefficient of variation
2	1.77	27.31 ± .58	14.47 ± .41	52.9 ± 1.9
4	3.30	29.32 ± .60	14.51 ± .42	49.8 ± 1.8
6	5.00	34.45 ± .65	15.46 ± .46	44.7 ± 1.6
8	6.68	34.20 ± .61	15.10 ± .43	44.1 ± 1.5
10	8.15	36.22 ± .72	16.93 ± .51	46.7 ± 1.7
12	9.72	34.31 ± .61	16.54 ± .43	48.2 ± 1.5
15	12.42	37.92 ± .66	15.96 ± .47	42.1 ± 1.4
20	16.69	37.07 ± .55	14.72 ± .39	39.7 ± 1.2
25	20.68	37.47 ± .49	15.39 ± .35	41.1 ± 1.1
35	28.85	39.43 ± .67	17.55 ± .47	44.5 ± 1.4
45	37.23	37.46 ± .51	15.19 ± .36	40.5 ± 1.1
55	44.65	40.04 ± .53	17.14 ± .37	42.8 ± 1.1
65	53.16	35.25 ± .45	15.97 ± .32	45.3 ± 1.1
75	59.66	32.34 ± .46	17.93 ± .32	55.4 ± 1.3
85	66.95	30.10 ± .36	15.06 ± .26	50.0 ± 1.0
95	74.50	27.17 ± .36	15.95 ± .25	58.7 ± 1.2
105	80.36	24.20 ± .32	14.87 ± .23	61.5 ± 1.2
125	94.38	19.60 ± .28	14.31 ± .20	73.0 ± 1.4
150	111.88	16.17 ± .24	13.75 ± .17	85.0 ± 1.7
200	144.47	11.93 ± .20	12.85 ± .14	107.7 ± 2.1

curve does not really represent a plateau of optimal density, in which region small differences in density make no significant difference in mean duration of life. After a density of 55 flies per bottle is passed the mean duration of life declines steadily with advancing density.

The form of the upper limb of the curve of decreasing mean duration of life with increasing density of population suggests that there is a tendency to approach a constant level or asymptote at extremely high densities. To test this point a further series of experiments was undertaken, with the results shown in Fig. 13. The general tech-

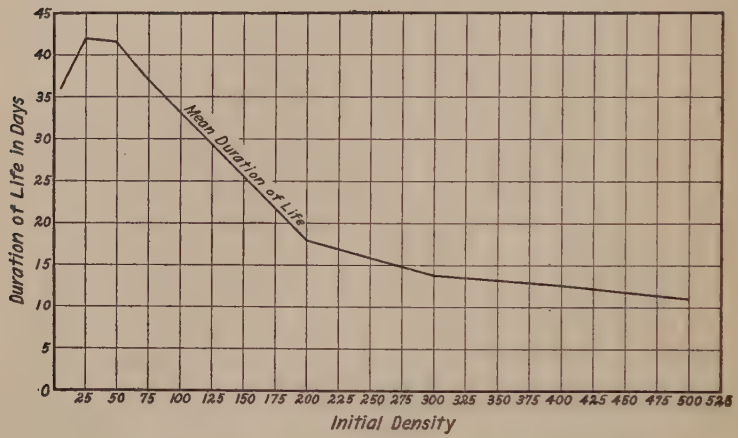


FIG. 13. Mean duration of life of wild type *Drosophila* at great densities of population.

nique of these experiments was identical with that of the earlier ones. The flies used were wild type, Line 107.

Allowing for the difference in the scale of plotting, it is evident that the experiments leading to Fig. 13 fully confirm the results of the earlier series. Furthermore Fig. 13 shows clearly the very gradual decline of mean duration of life with increasing densities of population after a den-

sity of 200 flies per one ounce bottle has been passed. Beyond this density little further effect on duration is produced by greater crowding.

The survivorship distributions at the extremely high densities approximate closely to the " straight diagonal line " type of life curve which has been discussed in Chapter III, and shown to be characteristic of vestigial flies. This is shown in Fig. 14.

The results so far described regarding the influence of density of population upon duration of life have been many times confirmed, by experiments conducted under a variety of conditions relative to other things than density of population. But always there results the same characteristic curve of mean duration of life shown in Figs. 12 and 13. Most of these experiments, however, were carried out with the old banana-agar food medium.

Inasmuch as the general ecological conditions are somewhat different in the bottles with the new synthetic food S–101 (96), from what they are with the old banana-agar medium, it seemed desirable to carry out density experiments with this new food medium. This we have done, with results entirely in accord with those already described. It is therefore justifiable to conclude that the density effects which have been observed depend on something other than the quality of the food upon which the flies live.

Having now shown the general nature of the relation between density of population and mortality there remains but one further series of experiments relative to this

environmental factor which needs to be discussed in connection with the development of our general argument.

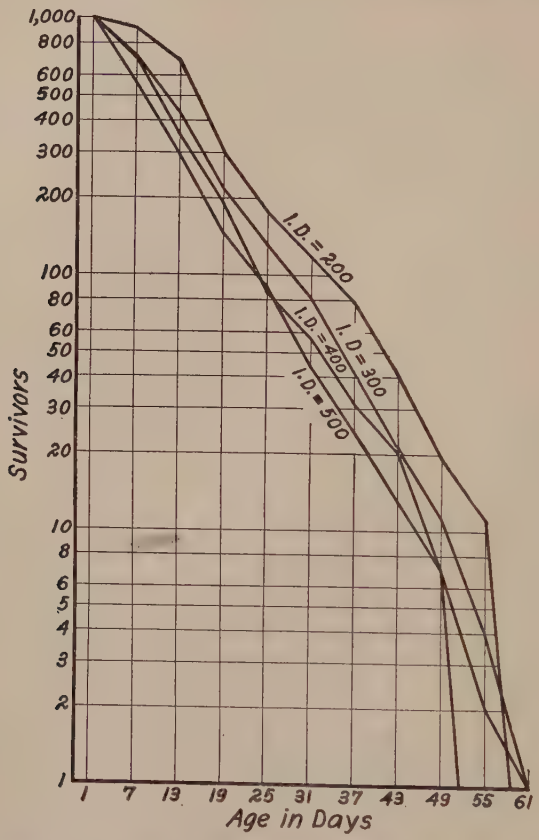


FIG. 14. Survivorship distributions for wild type *Drosophila* in densities of 200, 300, 400, and 500 flies per one ounce bottle. The abscissae are absolute ages in days.

What is the effect of sudden and large changes in population density during the course of the life of the flies? In all the experiments so far described the density has been allowed to change gradually merely as a result of the mortality itself. Suppose now we exercise the god-like privileges of the experimenter and alter suddenly the density.

It has been seen that a density of 35 flies per one ounce bottle is at least near the optimal density for duration of life. On the other hand a density of 200 flies is extremely deleterious to duration of life. Let us now set up a series of bottles according to the following plan.

A. Bottles at initial density 35, serving as controls, and not further manipulated except that on the morning of the 16th day of their age the then surviving flies in each bottle shall be etherized and the tips of the wings of a half of them clipped off with iridectomy scissors, the flies all being then returned to their proper bottles.

B. Bottles at initial density 200, serving as controls and not further manipulated, except that on the morning of the 16th day of their age they shall be etherized and counted.

C. Bottles at initial density 200, in which the mortality shall be allowed to go on at the normal rate for this density for 15 days. On the morning of the 16th day the number of flies in each such bottle shall be brought back to 200, by adding flies which up to that time have been in bottles of initial density 35 (optimal). Of the flies taken from bottles of initial density 35 to fill the 200 bottles back to

the density of 200, each shall have had the tips of its wings clipped off, so that it can be subsequently identified.

The case then stands this way. Flies starting at initial density 200 are to be allowed to die off in a manner normal for that density till they are 16 days old. Then the survivors are to be once more submitted to a density of 200 flies per one ounce bottle, the flies used to bring back the density to this point having been marked (by wing clipping) so that their subsequent mortality may be separately recorded. These added flies are also aged 16 days at the time they are subjected to a density of 200, but during the first 15 days of their lives they have been subjected only to the optimal densities implied by an initial density of 35 flies per bottle.

The experiments according to this plan were carried out with wild type Line 107 flies, on banana-agar food, at 25° C., the general technique being identical with that of the earlier experiments.

The survivorship distributions are given in detail in (98) and need not be repeated here. They are shown graphically in Figs. 15 and 16. The biometric constants for duration of life are given in Table 4.

The results of the experiments showed that, just as in the earlier work, the mean duration of life is much greater (in these experiments about double) among flies subjected to the conditions implied by an initial density of 35 flies per one ounce bottle than among flies subjected to the conditions implied by an initial density of 200. But when flies which have lived the first 15 days of their lives under the

TABLE IV

BIOMETRIC CONSTANTS FOR DURATION OF LIFE WITH CHANGED DENSITY OF POPULATION

Group	Mean (days)	Standard deviation (days)	Coefficient of variation (per cent)
A. Controls at initial density 35			
1. Total distribution.....	33.27 ± .41	13.27 ± .29	39.87 ± .99
2. Mortality from 16 days of age on. Wings not clipped	35.68 ± .54	11.67 ± .38	32.70 ± 1.18
3. Mortality from 16 days of age on. Wings clipped..	34.82 ± .54	12.28 ± .39	35.26 ± 1.24
B. Controls at initial density 200			
1. Total distribution.....	16.95 ± .27	11.23 ± .19	66.25 ± 1.60
C. Changed density.....			
1. Wings not clipped. Subjected to density 200 at age 1 day, and again at age 16 days. Mortality from 16 days on.....	19.71 ± .17	4.38 ± .12	22.24 ± .64
2. Wings clipped. Subjected to density 35 at age 1 day, and to density 200 at age 16 days	22.83 ± .19	7.27 ± .13	32.56 ± .66
3. Total distribution. Mortality from emergence on..	13.44 ± .14	6.05 ± .10	45.02 ± .85

conditions implied by an initial density of 35, are on the 16th day of their age submitted to a density of 200, and live out the remainder of their lives under the conditions implied thereby, their average duration of life is reduced in these experiments from the 34 or 35 days which it

would have been had they stayed in the bottles of initial density 35, to 22.83 days. This result shows that crowding produces a heavy increase in mortality even though it occurs as late as 16 days of age.

Flies which have lived for the first 15 days of their lives under the conditions implied by an initial density of 200, and then at the age of 16 days are again subjected to a density of 200, have a significantly shorter duration of life than do their companions in the same bottles which spent the first 15 days of their lives in bottles of initial density 35. The difference is $22.83 \pm .19 - 19.71 \pm .17 = 3.12 \pm .25$ days. This difference is more than 12 times its probable error. It may be taken as probable to the point of practical certainty that excessive crowding in early life deleteriously affects the survivors at 16 days of age, so that they are significantly less resistant to the effects of heavy crowding again at that time than are flies which lived at optimal densities in early life.

Clipping the wings to the extent which was practiced in these experiments for purposes of identification did not significantly affect duration of life ($35.68 \pm .54 - 34.82 \pm .54 = 0.86 \pm .76$, an obviously insignificant difference).

The course of events in the experiments is shown graphically in Figs. 15 and 16.

Figure 15 shows that when the flies which had spent the first 15 days of life in bottles of initial density 35 were crowded up to density 200 their survivorship curve at once dropped to the level of the 200 density control bottles and continued nearly to the end of life parallel and

very close to that curve. Those that started at density 200, when brought back to this density at 16 days of age

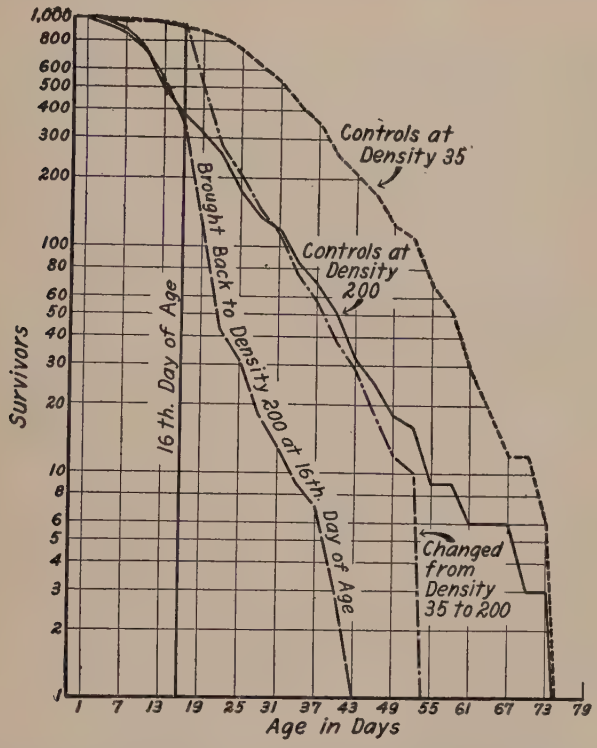


FIG. 15. Survivorship curves showing the effect of changing density of population at the 16th day of age. Explanation in text.

sustained at once a great increase in mortality, and their survivorship curve dropped and stayed well below the 200

density control curve, throughout the remainder of the life span.

Figure 16 demonstrates, in the first place, that clipping

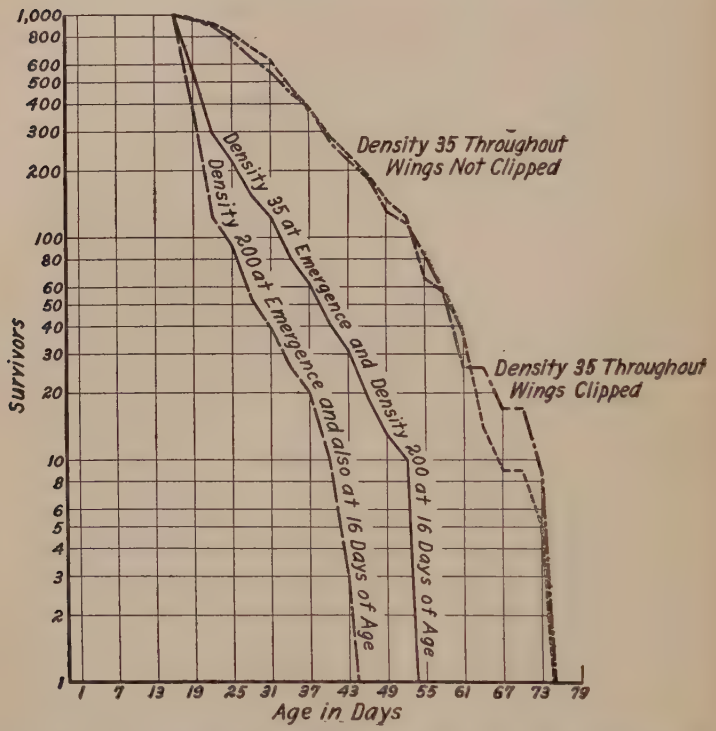


FIG. 16. Survivorship curves starting with 1000 individuals at age 16, showing the effect of prior history upon duration of life after that age.

the tips of the wings to the extent practised in these experiments did not *per se* alter the rate of mortality in those

flies that remained in bottles of initial density 35 throughout life. The two upper survivorship curves could hardly be closer together than they are. Furthermore this diagram shows in a striking way the fact that flies which had spent the first 15 days of life at high densities were much less able to withstand the deleterious effect of renewed high densities, beginning at age 16 days, than were flies which had spent the first 15 days of life at optimal densities. It might have been thought, *a priori,* that the heavy mortality at the start in the bottles of 200 initial density would have been selective, and its survivors better able to withstand later environmental strain. The results, however, do not support this hypothesis.

There is a small literature of careful experimental studies on the effect of density of population upon different biological processes, notably growth. The more important papers and books are those of Semper (125), Bilski (6), Drzwina and Bohn (22), Pearl and Surface (114), Pearl and Parker (102), Pearl, Allen and Penniman (96), Pearl (92), de Varigny (19), Robertson (119, 120), Greenleaf (34), Cutler and Crump (14–16), Legendre (61), and Willem (137).

There is also a statistical literature on the correlation between density of population and mortality in human populations. Here the important references are Farr (25), Brownlee (11, 12), LeBlanc (60), Mortara (73), Bowley (9).

There can be no question that the degree of crowding of organisms together in spatially limited " universes "

has a profound effect upon various vital processes. In this chapter it has been shown that different degrees of density of population have associated with them marked and orderly differences in the duration of life of *Drosophila melanogaster*. In all of the experimental work there has been found no other environmental factor which produces such marked alterations in life duration. The discovery that high degrees of crowding shorten life (increase the rate of mortality) is the result to be expected on general grounds. But the further clean-cut evidence that minimal population densities are not optimal for duration of life in wild type *Drosophila* is an unexpected result.

The experiments also suggest that there is a limit to the effect upon duration of life which can be produced by increasing the degree of crowding of the flies in the bottle. After a density of 200 flies per one ounce bottle is reached further increases in density of population produce but slight further reductions in mean duration of life.

It has further been shown that the most marked effect of density of population upon life duration is produced early in life, though excessive crowding can immediately increase the death rate at later periods of life, and by inference at any age. It also appears that the amount of shortening of life produced by crowding at any age is influenced by the previous history of the flies relative to density of population. This suggests that there is a deleterious biological effect of crowding in early life even upon those flies that do not immediately die as a result of

it, and that this effect endures for at least the first 15 days of life.

It has been seen in this chapter that an environmental factor, density of population, is able, if forced to something approaching the limit, to convert the characteristic life curve of normal wild type *Drosophila* shown in Chapter III into the form which is normal for the mutant vestigial fly. But plainly this density of population factor cannot be the explanation of the appearance of the diagonal line type of survivorship curve in normal vestigial populations, because these curves are obtained in such populations at *optimal* densities. The explanation must be sought elsewhere, and the suggestion is obvious that it may be found in the genetic constitution of vestigial flies. What, in general, is the influence of heredity in determining duration of life? Let us next examine the evidence on this point.

CHAPTER V

INHERITANCE OF LONGEVITY

From experiments and observations upon *Drosophila* two significant lines of evidence have been obtained as to the inheritance of duration of life. The first of these consists in the proof that there exist in a general population of *Drosophila melanogaster* (or its mutants) genetic differences in respect of duration of life. It can further be shown that these differences are capable of isolation by appropriate selection and inbreeding, and that within an even moderately inbred line, the genetic differences in duration of life remain constant over periods of at least ten to twenty-five or more generations. For complete details of the work the reader must consult the original paper (100). Here there can be presented only a brief résumé.

In the case of an organism like *Drosophila* it is plainly impossible to have a pure-line in the strict sense of Johannsen (53). The most that one can do is to have inbred lines, and the most intense degree of inbreeding possible for bisexual animals is by brother x sister mating. The general plan of the experiments to determine whether genetic differences in duration of life exist in general populations, may be outlined as follows:

1. Mate a virgin brother and sister, chosen at random from a given stock.

2. Repeat this for as many pairs as possible.

3. Test the progeny of each mated pair separately for duration of life, and form for each group of such progeny a life table.

4. Each such mated pair constitutes the beginning of a line, in which at any time the processes noted under paragraphs 1, 2 and 3 above can be repeated, and in fact were.

TABLE V

FREQUENCY CONSTANTS FOR DURATION OF LIFE. FIRST TEST

Line No.	Mean Duration of Life (Days)	Standard Deviation (Days)	Coefficient of Variation
100	40.45 ± 0.84	16.38 ± 0.59	40.49 ± 1.68
101	50.02 ± 0.85	15.51 ± 0.60	31.01 ± 1.31
201	47.40 ± 0.99	15.03 ± 0.70	31.71 ± 1.51
202	22.04 ± 1.57	18.18 ± 1.11	82.49 ± 7.74

Table 5 gives the biometric constants for the first of such tests, the progeny of the matings of single brother x sister pairs, the mated siblings being the offspring of individuals taken at random from long-lived wild type stocks.

From these data it is at once apparent that these progeny groups show distinct, and in some cases decidedly large, differences both in mean duration of life and in its variability. Lines 101 (Old Falmouth stock) and 201 (New Falmouth stock) show the longest mean durations

of life. The difference in the means for these two lines is 2.62 ± 1.31 days, probably an insignificant divergence. Similarly these two lines do not significantly differ in absolute or relative variability, the difference between the standard deviations being 0.48 ± 0.92.

Line 100 (Old Falmouth stock) has a distinctly and significantly lower mean duration of life than 101 or 201. Comparing it with line 101 the difference in the means is 9.57 ± 1.20 days, a difference approximately 8 times its probable error. Line 100 is also relatively more variable in duration of life than 101 and 201.

The individuals in line 202 (New Falmouth stock) are the shortest lived of any here dealt with, and the shortest lived wild type strain isolated. The mean duration of life is less than half that shown by lines 101 and 201 and only a little more than half that of line 100. Line 202 shows the highest relative variability in duration of life of any of the lines. It also has the highest absolute variability.

During the progress of the experiments just described the offspring flies (from original brother x sister matings) from each of the lines in which duration of life was being tested, were allowed to mate at random in their bottles, and their progeny removed to form stocks of the several lines. These stocks were allowed to reproduce in stock bottles, all matings being therefore random within the line, for a period of about 7 months. At the end of that time it was decided to make a retest of each line to see how it was then behaving relative to duration of life. Two individuals, a male and a female, were taken at random

from each stock bottle. From these brothers and sisters were bred, and pairs of such siblings were mated to get sets of progeny on which to carry out a second series of life duration experiments.

The purpose of this second test was to determine the extent to which duration of life was holding constant in the line. During the period between the first and second test the stocks of the several lines had been subjected to varying environmental influences, in particular in relation to temperature, the stock bottles having been kept at room temperature, which varied rather extensively. Did the lines after 7 months have the same characteristic life curves that they exhibited on the first test? Allowing 12 days to a generation as a rough average figure in the case of flies reproducing freely at random in stock bottles, the interval elapsing between the first and second tests would cover approximately 18 generations. This is a long period biologically and affords abundant opportunity for change in the average genetic constitution of the population.

TABLE VI

FREQUENCY CONSTANTS FOR DURATION OF LIFE. SECOND INBRED TEST

Line No.	Mean Duration of Life (Days)	Standard Deviation (Days)	Coefficient of Variation
104	39.59 ± 0.74	18.63 ± 0.53	47.06 ± 1.62
107	53.74 ± 1.07	17.40 ± 0.75	32.38 ± 1.54
207	45.34 ± 1.10	19.97 ± 0.78	44.04 ± 2.03
208	25.65 ± 1.53	14.68 ± 1.08	57.23 ± 5.42

An examination of Table 6 shows at once, in a general way, that the characteristic features of the several lines in respect of duration of life did in fact remain remarkably constant during this period.

The experiments so far described establish two significant points in our analysis of the mortality phase of group biology. The first is that in a general laboratory population of *Drosophila* there exist genetically diverse individuals. If these individuals are isolated and inbred they can be multiplied up into strains or lines which exhibit characteristic and marked diversities, as groups, in respect of duration of life. The second point is that if the isolation of the lines so established is maintained, and individuals are permitted to mate only within the line, the characteristic diversities in duration of life are maintained approximately constant over long periods, biologically speaking.

These results have been many times confirmed in my laboratory, and they constitute a sound basis for the conclusion that genetic factors control or determine duration of life, in part at least, in *Drosophila*.

The fact shown by the life tables discussed in Chapter III that mutant flies differing from the normal wild type only in the wing character vestigial have a duration of life only approximately one-third that of the normal wild type, obviously suggests the appropriate next step in the experimental analysis. It is to breed these two kinds of flies together, and follow by the usual Mendelian technique the behavior of the offspring in the subsequent gen-

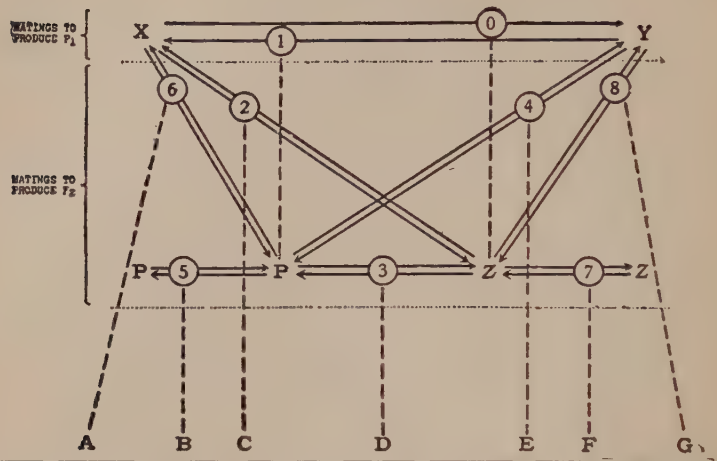
erations, in respect of life duration. This study has been carried out on an extensive scale (106 where full numerical details will be found). In these experiments the flies used as the short-lived parent stock in the matings carried, in addition to the wing mutation vestigial, four other mutant characters, " black," " purple," " arc," and " speck." These mutations are described in Bridges and Morgan (10). The quintuple stock had substantially the same mean duration of life and form of life curve as pure vestigial stock.

It will be convenient to designate the matings in these Mendelian experiments according to a standard plan devised some years ago (84), and reproduced here in Table 7.

In explanation of this table it should be said that letters denote *individuals,* or groups of individuals which are brothers and sisters. Solid lines, with circles in their course, connecting letters, denote matings of the kinds of individuals indicated by the connected letters. A dotted line leads from the mating to the kind of individual produced. Arrow heads indicate the direction of the mating, the arrow being supposed always to pass from the male to the female. Separate numbers are not given to reciprocal matings after the matings of the original parental generation to produce the first hybrid generation, F_1. To designate separately reciprocal matings after that point would greatly complicate the system without any significant gain from a practical point of view. The numbers within the circles are the designations (or names) of the

TABLE VII

MATINGS TO PRODUCE F_3



F_2 Individuals Mated	Number of Mating	F_2 Individuals Mated	Number of Mating	F_2 Individuals Mated	Number of Mating	F_2 Individuals Mated	Number of Mating
$A \times X$	10	$B \times Z$	46	$C \times F$	51	$E \times E$	19
$A \times Y$	12	$B \times B$	13	$C \times G$	53	$E \times F$	45
$A \times P$	40	$B \times C$	37	$D \times X$	22	$E \times G$	47
$A \times Z$	42	$B \times D$	29	$D \times Y$	24	$F \times X$	30
$A \times A$	11	$B \times E$	55	$D \times P$	52	$F \times Y$	32
$A \times B$	33	$B \times F$	57	$D \times Z$	54	$F \times P$	60
$A \times C$	25	$B \times G$	59	$D \times D$	17	$F \times Z$	62
$A \times D$	35	$C \times X$	18	$D \times E$	43	$F \times F$	21
$A \times E$	61	$C \times Y$	20	$D \times F$	31	$F \times G$	49
$A \times F$	63	$C \times P$	48	$D \times G$	27	$G \times X$	34
$A \times G$	65	$C \times Z$	50	$E \times X$	26	$G \times Y$	36
$B \times X$	14	$C \times C$	15	$E \times Y$	28	$G \times P$	64
$B \times Y$	16	$C \times D$	39	$E \times P$	56	$G \times Z$	66
$B \times P$	44	$C \times E$	41	$E \times Z$	58	$G \times G$	23

matings, and from the very nature of the case, these numbers designate not alone the particular mating but also, in the first hybrid and later generations, the nature of the pedigree of each of the individuals entering that mating.

1. All even numbers refer to back-cross matings.
2. All odd numbers refer to co-fraternal or intra-generation matings (not back-crosses).
3. Matings below 2 are of parental generation individuals: between 2 and 8 inclusive are of individuals of the first hybrid generation; matings over 10 are of individuals of the second generation from the original cross.
4. Even numbers from 10 to 36 inclusive designate back-crosses of individuals of the second hybrid generation with their grandparents, or individuals of the grandparental generation.
5. Even numbers from 40 up designate back-crosses of individuals of the second hybrid generation on individuals of the first hybrid generation.

When the long lived wild type stock is crossed with the quintuple short lived stock the results in the offspring generation F_1 are shown in Table 8, and Fig. 17.

The first generation progeny directly from the cross are structurally like the wild type parent. The morphological mutations carried by the quintuple flies behave as Mendelian recessives to their wild type allelomorphs. Also the duration of life in the first generation flies is essentially similar to that established by their wild type parent. They

TABLE VIII

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF PARENTAL AND F_1 FLIES

Group	Mating Type	Mean Age at Death	Standard Deviation	Coefficient of Variation
Parental Old Falmouth. Wild	—	44.26 ± .44 days	16.57 ± .31	37.44 ± .80
Quintuple.....	—	14.08 ± .23 days	6.93 ± .16	49.23 ± 1.43
Quint. ♂ × Old Fal. ♀	(0)	51.73 ± .57 days	15.71 ± .40	30.37 ± .85
F_1 Old Fal. ♂.. × Quint. ♀.	(1)	51.12 ± .84 days	15.29 ± .59	29.90 ± 1.26
All F_1's.....	(0)+(1)	51.55 ± .47 days	15.58 ± .33	30.23 ± .70

are long-lived flies. In actual fact the first generation flies have an even longer mean duration of life than the long-lived, wild type parent. Taking all the first generation progeny flies together the difference in the mean over the wild type parent form is $51.55 - 44.26 = 7.29 \pm 0.64$ days. This is a certainly significant difference amounting to 11.4 times its probable error. The survivorship lines in Fig. 17 show plainly the superior longevity of the first generation flies over the longer-lived parent. This phenomenon is objectively similar to the enhanced vigor observed in the first generation plants in crosses of maize, as described by East and Jones (23), Shull (126) and others. It probably has the same explanation.

There is no significant difference in respect of duration of life between the reciprocal crosses. The mean duration

of life, and the variability are identical whether the cross-bred individuals had a wild-type father or mother. The

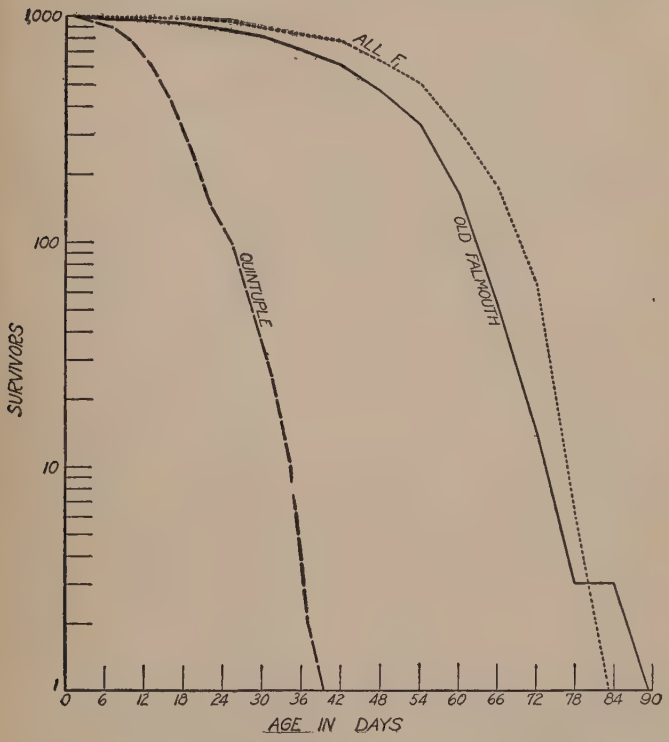


FIG. 17. Observed survivorship distributions for parental (wild type and quintuple) and F_1 flies.

first generation flies are significantly less variable relatively in duration of life than are either of the parent stocks.

From this point on the matter necessarily gets more complicated. In order not to encumber the text with numerical details the tables of biometric constants for the duration of life of the second and third generation flies are relegated to Appendix II of this book. Here in the text the essential results may be most simply presented by means of pedigree diagrams.

When flies carrying the mutation vestigial are crossed with normal, wild type flies, the wing mutant character vestigial behaves as a simple Mendelian recessive, without sex-linkage since the gene for vestigial is located in the second chromosome. The first generation flies from such a cross are all of the normal wild type, with normal wings. When these first generation flies are mated with each other, they produce a second generation (second from the original cross) in which individuals bearing normal wings, and individuals bearing vestigial wings occur in the theoretical proportion of three of the former to one of the latter. The actually realized proportions approximate to this theoretical ratio.

These elementary facts are graphically shown in Fig. 18.

Turning now to the facts regarding duration of life Fig. 18 shows what has already been brought out above, that for the first generation flies, which are all of normal wild type morphologically, the mean duration of life is approximately 52 days. When these flies are bred with each other the wild type flies of the second generation have a mean duration of life of 46 days, while the reces-

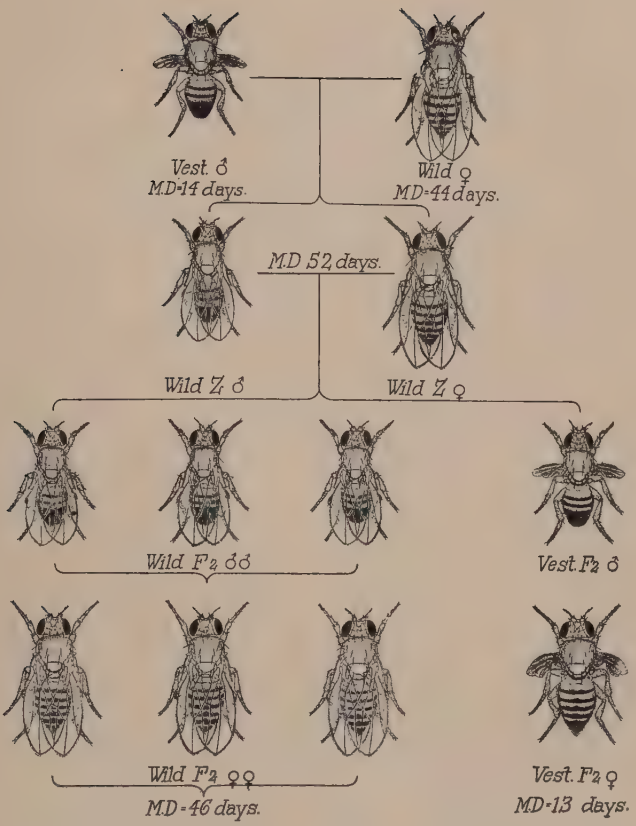


FIG. 18. Pedigree diagram showing the results in the first two generations of crossing a vestigial mutant with a normal wild *Drosophila*.

sive vestigial flies of this second generation have a mean duration of life of only 13 days. There is thus a clean segregation in respect of average longevity exactly paralleling that in the morphology of the wings. The forms segregated in the second hybrid generation have the same average duration of life as the corresponding original parent forms, to within one or two days.

Fig. 19 shows that the same result is obtained if the original cross is made in the reciprocal direction, that is

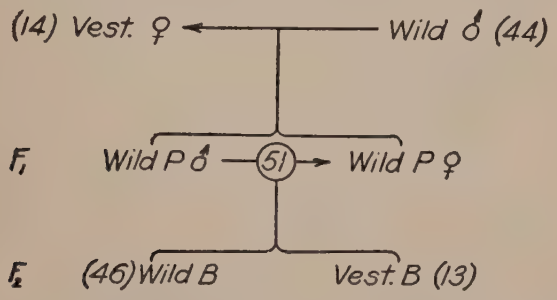


Fig. 19. Pedigree diagram of reciprocal cross to that shown in Fig. 18.

the wild type male on the vestigial female. In Fig. 19 and the following diagrams the figures in brackets or circles denote mean duration of life in days, and the letters and subscript figures refer to mating types designated in Table 7.

There is one further sort of *inter se* mating of first generation individuals, namely that involving the breeding of males of the *Z* type (Table 7), with females of the *P*

type, and the reciprocal of this. The results of these matings are shown in Figs. 20 and 21.

The results shown in Figs. 20 and 21 are generally the same as those of Figs. 18 and 19. Again there is in the second hybrid generation a segregation in respect of dura-

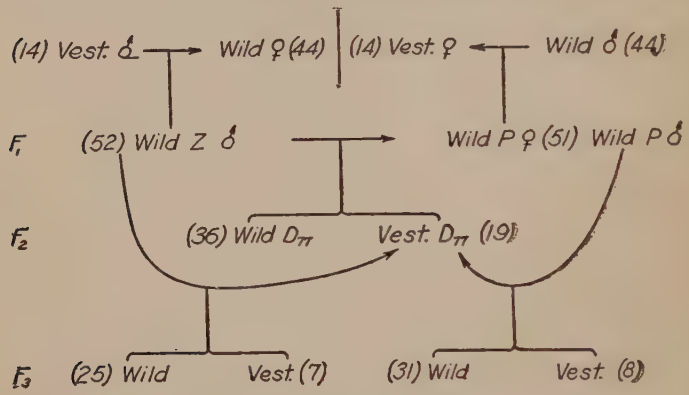


FIG. 20. Diagram showing the results of breeding F_1 males of pedigree type *Z* with F_1 females of pedigree type *P*, and of back-crossing the F_2 vestigial segregates to their F_1 parent forms.

tion of life. There is, however, a difference of detail which is worth noting, though I have no explanation to offer for it. The *inter se* matings of first generation individuals of the type *P* x *P* and *Z* x *Z* give uniform results. So also do the matings of type *P* x *Z* or *Z* x *P*, when compared with each other. But these latter mating types give consistently different results from either *P* x *P* or *Z* x *Z*. The normal winged flies of the second hybrid generation from the *P* x *Z* (or *Z* x *P*) type of *inter se* first generation matings

are significantly *shorter-lived* than the corresponding second generation flies from $P \times P$ (or $Z \times Z$), and the second generation vestigial flies from $P \times Z$ (or $Z \times P$) are significantly *longer-lived* than the corresponding recessive segregates from $P \times P$ (or $Z \times Z$).

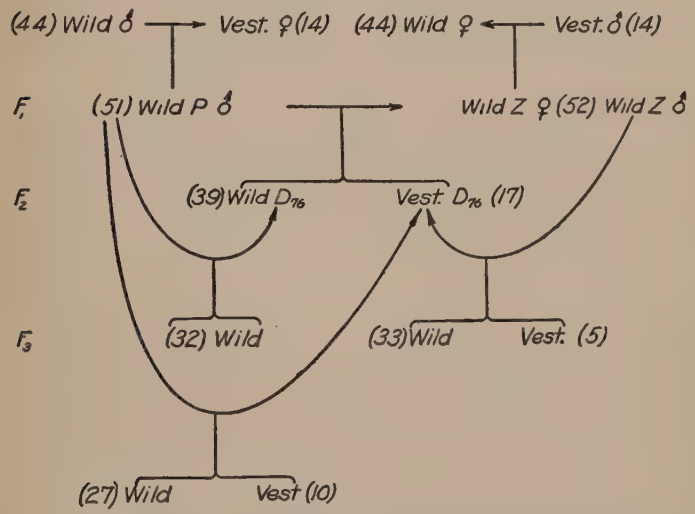


FIG. 21. Diagram of reciprocal cross (in F_1) to that shown in Fig. 20.

Figures 22 to 29 inclusive show the results of various experiments in back-crossing first generation individuals. The nature of the matings is apparent from the diagrams and their legends.

The general result of the back-cross matings is again to show indubitably a segregation in respect of duration of

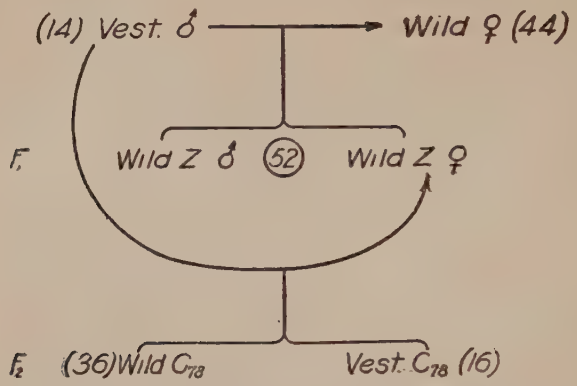


FIG. 22. Results of back-crossing an F_1 female of pedigree type *Z* with a parent generation male pure for vestigial.

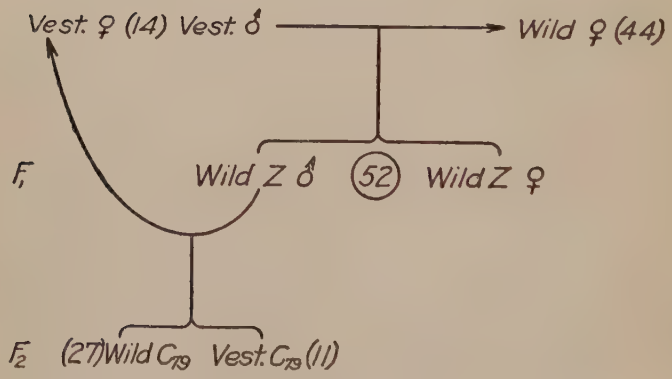


FIG. 23. The reciprocal back-cross to that shown in Fig. 22.

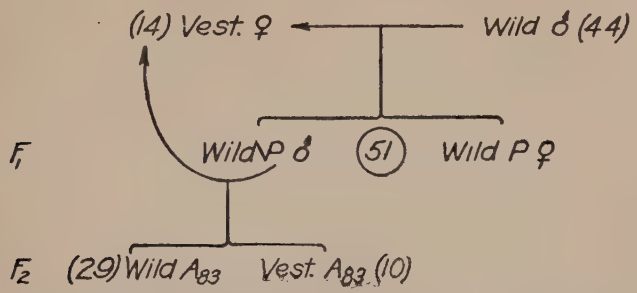


FIG. 24. Results of back-crossing an F_1 male of pedigree type *P* with a parent generation female pure for vestigial.

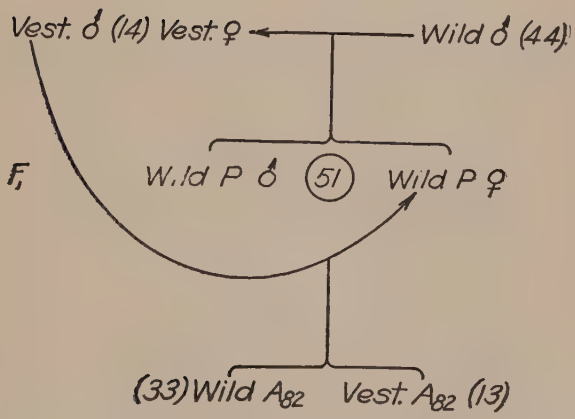


FIG. 25. The reciprocal back-cross to that shown in Fig. 24.

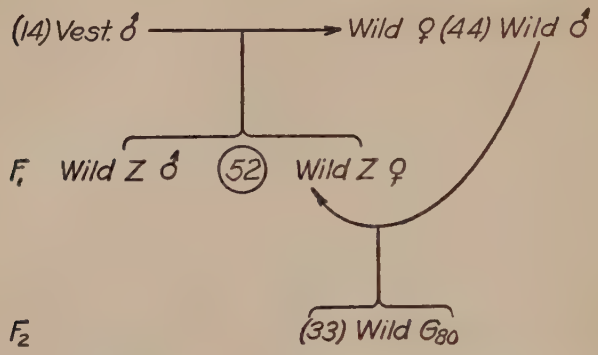


FIG. 26. Results of back-crossing an F_1 female of pedigree type *Z* with a normal wild type male.

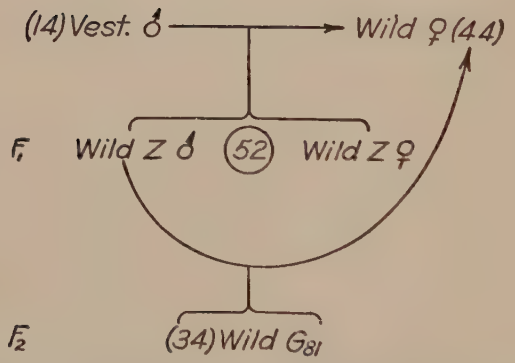


FIG. 27. The reciprocal back-cross to that shown in Fig. 26.

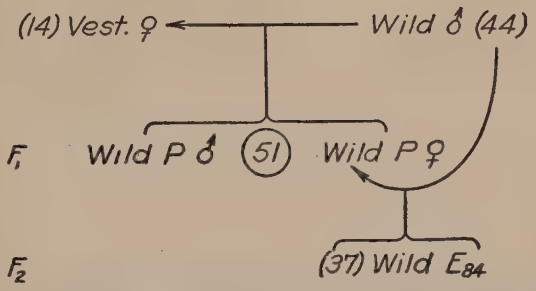


FIG. 28. Results of back-crossing an F_1 female of pedigree type *P* with a normal wild type male.

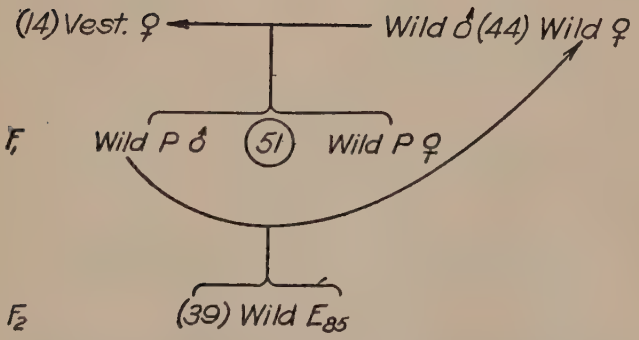


FIG. 29. The reciprocal back-cross to that shown in Fig. 28.

life, but with certain differences in detail, as compared with *inter se* matings of first generation flies. For discussion of these details the original (106) must be consulted.

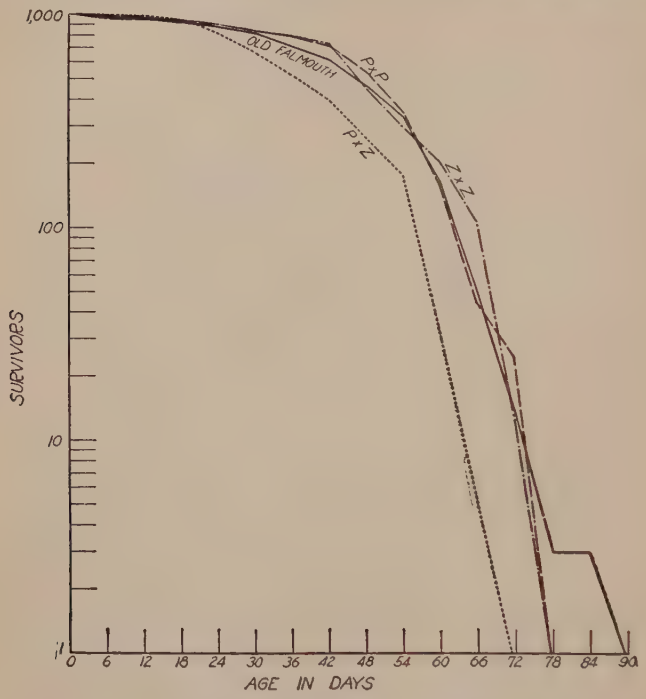


FIG. 30. Survivorship lines for F_2 wild type flies from F_1's mated *inter se*. Dash line = $P \times P$; dot line = $P \times Z$; dash-dot line = $Z \times Z$; solid line = original wild type parent stock.

In all the matings so far considered there is one broad result which is apparent besides the evident segregation in respect of duration of life. It is that there is a general

tendency towards a degradation in average longevity in second generation flies, as compared with those of earlier generations. This tendency is continued in the third

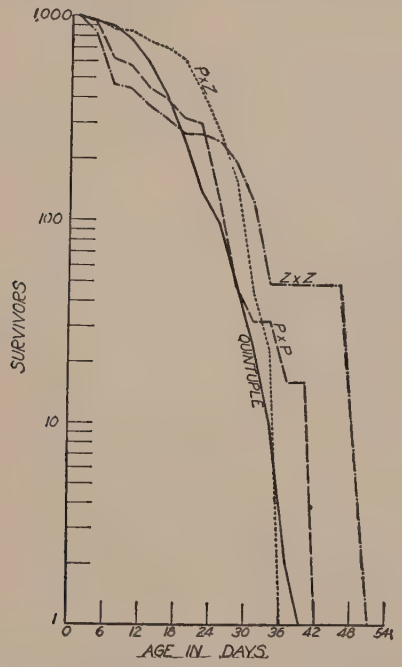


FIG. 31. Survivorship lines for F_2 vestigial flies from F_1's mated *inter se*. Dash line = $P \times P$; dot line = $P \times Z$; dash-dot line = $Z = Z$; solid line = original vestigial parent stock.

hybrid generation as shown in Tables 26 and 27 of Appendix II. The biological significance of this result will be discussed in a later chapter.

Summing the whole case up it is seen that duration of life behaves in the first, second, and all that were tested of the possible third generations of offspring from reciprocal crosses of short-lived and long-lived strains of *Drosophila* as any character of the organism would be expected to behave if it were inherited in a Mendelian manner. There is a somewhat higher duration of life in the first hybrid generation than in either of the parent stocks (effect of heterosis). There is a clear-cut segregation of long-lived and short-lived groups in subsequent generations.

In its genetic behavior, duration of life is completely and invariably associated with certain morphological characteristics of the flies, in the sense that no vestigial-winged fly was found in these experiments to be long-lived, and no group of normal-winged flies has ever been found to have a life curve even approaching in form that which was characteristic of the vestigial-winged flies.

Since there is this invariable association between duration of life and the morphology of the wing it might be argued that perhaps the reason why vestigial winged flies are invariably short lived is merely and directly physiological, arising because such a fly has no functional wings and therefore can not fly, but has its activity confined to walking or jumping. Stated in extreme forms, the idea would be that so far as inherent constitutional factors influencing duration of life are concerned quintuple and wild stocks are on the same footing, but because the quintuple fly has no functional wings it is physiologically un-

able so to conduct its life as to realize its constitutional potentialities in respect of longevity.

It is possible to test this idea experimentally, in part at least. Suppose in a large number of wild type flies immediately after emergence the wings are cut off close to the body. Since they do not regenerate, these flies must then go through life wingless. What difference in the distribution of mortality of *Drosophila* will the absence of wings make in a purely physiological, somatic sense?

In the actual experiments to test the point the surgery was rather rough and ready. The flies were etherized immediately after emergence and the wings clipped off as close as possible to the body with iridectomy scissors, care being taken to avoid injuring the *halteres*. No attempt was made to seal the wounds and there was visible loss of body fluids. The post-operative mortality was rather heavy, but Table 9 shows that after 40 days of age the age specific death rates were not essentially different in the operated flies than in the normal controls.

The total death rates, over the ages covered, differ by only 3.98 ± 4.26, an obviously insignificant amount.

Following the experiments just described Gonzalez (33), in my laboratory, carried out an extensive investigation of the effect upon duration of life of each of the five mutant genes black, purple, vestigial, arc, and speck. The mutant gene speck definitely and significantly has associated with it an increased duration of life. Flies differing from wild only in the possession of the gene for black have

TABLE IX

DEATH RATES OF (*a*) WILD TYPE DROSOPHILA WITH WINGS SURGICALLY REMOVED AT EMERGENCE, AND (*b*) NORMAL CONTROLS

Age in days	Wings surgically removed			Normal Controls		
	Fly-days exposed to risk	Deaths	Rate 1000 m_x	Fly-days exposed to risk	Deaths	Rate 1000 m_x
40–42	591	21	35.53	918	33	35.95
43–45	523	26	49.71	818	34	41.56
46–48	447	24	53.69	716	34	47.49
49–51	379	20	52.77	615	33	53.66
52–54	307	32	104.23	507	42	82.84
55–57	215	28	130.23	359	64	178.27
58–60	132	27	204.55	206	25	121.36
61–63	67	11	164.18	136	20	147.06
64–66	36	9	250.00	84	12	142.86
67–69	17	1	58.82	55	5	90.91
70–72	12	3	250.00	37	8	216.22
73–75	5	1	200.00	19	2	105.26
76–78	2	1	500.00	11	4	363.64
79–81	....	...	.....	3	...	0
82–84	....	...	.....	2	1	500.00
Totals	2733	204	74.64 ± 3.39	4486	317	70.66 ± 2.58

the same average duration of life as normal wild flies. Each of the other three genes has associated with it some reduction of mean longevity as compared with that of normal wild flies. In flies carrying different combinations of these five genes the duration of life is not generally the average of the durations associated with the single genes involved, when these are present alone in pure form.

Furthermore the total number of mutant genes present (within the range of the five studied) has no relation to the duration of life of the fly.

We have, taking all this experimental work together, a large mass of clean-cut facts regarding duration of life in groups of individuals of known inheritance, which demonstrates a definite relationship between genetic constitution and longevity. How are these facts to be interpreted?

The most reasonable interpretation, consonant with all the facts, seems to be to assume that duration of life, considered of itself, is not a biologically separate characteristic of the organism, but instead is simply the expression of the total functional-structural organization or pattern of the individual. It is this organization or pattern which is inherited, and not duration of life as such. Duration of life is only one of many objective manifestations of the thing which is inherited. Put in another way, what this view of the matter says is that if the duration of an individual's life is an implicit function of the individual's organization or constitution (presumably a purely physical and chemical thing fundamentally) and if constitution or organization is inherited, it follows that duration of life will behave exactly as though it were itself inherited.

In the experiments here described it has been shown that the gene for vestigial, besides affecting the form, size and behavior of the wings of *Drosophila, also* influences in and through its effects on the total organization pattern of every fly in which it is present, the duration of life of

that fly. We have measured with considerable exactness and manifoldness what the quantitative effect of this gene is upon duration of life (and, of course, at the same time the effect of its allelomorph the gene for normal wing).

This view of the case seems to be in accord with the best current opinion as to the biological meaning of mutant genes generally. Thus Morgan, in his latest book (71, p. 304) says: "It must not be supposed, however, from what has just been said, that mutant changes produce only a single striking or even a single small change in one particular part of the body. On the contrary, the evidence from the *Drosophila* work, which is in accord with that from all other forms that have been critically studied, shows that even in those cases where one part is especially modified, other effects are commonly present in several or in all parts of the body. The subsidiary effects not only involve structural modifications, but physiological effects also, if one may judge by the activity, the fertility, and the length of life of the mutants. For example, the loss of positive phototropism, characteristic of *Drosophila*, accompanied a change involving a very slight alteration in the general color of the body."

Recently Dobzhansky (20) has developed this view and supported it by extensive *ad hoc* experimental evidence from *Drosophila*.

It has lately been shown by Smith (127) that in the white sweet clover, *Melilotus alba*, the annual habit of growth, as contrasted with the normal biennial habit, is

controlled in inheritance by a simple gene. He interprets the difference between the two habits of growth as fundamentally a difference in duration of life. On this interpretation the case parallels the effect in *Drosophila* of the mutant gene vestigial, particularly as Smith notes various structural differences, notably in cell size, between the annual and biennial forms.

The experimental data presented in this chapter advance our understanding of the mortality phase of the biology of groups by showing that the death rates of a group of animals (or any derivative function of these death rates such as the mean duration of life) are related in a definite manner to the genetic constitution of the individuals composing the group. This relationship is of so precise a character in these experiments that if the genetic constitution of two individuals is known, within the range of genes so far analyzed in this respect, the average duration of life of their progeny can be predicted to a close degree of approximation, it being understood that the environmental conditions are constant and substantially optimal, as they were in these experiments. The notion that life duration is influenced in some degree by heredity has received statistical support from human experience. So far as one organism, *Drosophila melanogaster,* is concerned we can now add experimental demonstration, quantitatively evaluated, to the purely statistical evidence.

But these results in turn set a further problem. What is it that is inherited? It has been suggested in this chapter

that duration of life is to be regarded as the integrated dynamic expression, in time units, of the organization, in the Aristotelian sense, of the individual, and that it is this organization which is inherited. Let us turn to more detailed examination of this conception in the next chapter.

CHAPTER VI

INHERENT VITALITY

THE conception of the biological basis of genetic differences in duration of life set forth summarily in the preceding chapter derives support from sources other than the *Drosophila* experiments so far recounted. In particular certain facts regarding human mortality (87, 91) are most reasonably interpreted on the hypothesis that duration of life is the expression in time of the genetically determined organization of the individual. But the solid grounding of the hypothesis must come finally from experimental investigations, rather than from any purely statistical analysis of human mortality data. In this chapter I shall endeavor to develop the hypothesis further. As a preliminary it is necessary to discuss briefly some basic concepts of biology.

However difficult it may be to define life satisfactorily, that is to say rigorously and comprehensively, there is no great practical difficulty in telling whether a particular aggregation of matter, spatially and temporally discrete, is in fact living or not-living or historically belongs in one or the other of these categories. There are many different ways in which we pragmatically make this discrimination,

which I need not stop to list. It is only necessary to suggest their categorical disposition by such words as movement, metabolism, permeability, etc. But all these ways depend ultimately upon the fact that the living organism does things which are different, and does them in ways which are different, from the activities of non-living aggregations of matter (cf. Leathes, 59). In short, the basis of the discrimination between living and not-living material rests upon *events* or *actions* rather than upon ultimate static structures. The essence of life lies in its dynamics and not in its statics.

The considerations just stated imply at once what is an easily observable fact, that there are differences in degree of intensity of the actions which distinguish living from not-living things. These differences may be noted on either a space or a time basis of reference or on a combined base which includes both space and time. Thus the degree of intensity of action may be different in each different individual organism at the same time in the group considered, or it may be different at different times in the history of the same individual.

It will be convenient in the discussion which is to follow to use the term *vitality*, in a general sense, to mean the degree of intensity of vital actions. Since this is a quantitative concept it is necessary to proceed at once to the consideration of its measurement. In the broadest terms such measurement will always finally depend upon the rate of energy transformation of the organism within defined limits in respect of space and time. Any functions involv-

ing this rate, such as the sum of energy stored plus energy set free, as recently suggested by Murray (75), may be used as a measure of " aliveness " or vitality. It may be that one will choose to measure the rate of metabolism or the rate of growth or the rate of movement or some other aspect of the energy transformations by the organism.

It should be clearly understood that vitality as here defined has no relation to or connection with the concept of " vitalism." It is the generally accepted view of biologists that the " activities of living organisms " are completely determined by purely physical and chemical mechanisms and forces, even though it is not possible in the present state of knowledge always to describe these mechanisms or to define these forces rigorously and particularly. The present discussion fully accepts this physico-chemical hypothesis. In fact its purpose is to apply to certain biological phenomena some of the methods of reasoning which have in recent years been so fruitful in the domain of physics. But in the discussion which is to follow it will be necessary to speak about " the degree of intensity of the activities of living organisms " frequently. The single word " vitality " is certainly less cumbersome to use than the detailed definition, and, the sense in which it is to be used having been defined, its use is not less exact or precise.

In the energy transformations of the living organism there is involved an irreversible progression which includes three elements, as follows:

A ——————> B ——————> C

External Sources of Energy and Non-Living Matter	*The Organism*	*The Product*
(Food. Either chemical compounds of external origin which yield energy and utilizable material when split by the organism (animals), or similar compounds synthesized by the organism itself, with energy derived from light, and subsequently split to yield energy and utilizable material.)	(An aggregation of matter which has the property of autonomously changing A to C, *by virtue of its pattern or organization.*)	(Either (a) heat, (b) work, (c) component matter of the organism, or (d) waste, meaning chemical compounds incapable of yielding energy with economic profit by further splitting.)

This *schema* represents, of course, in another form, Cuvier's "whirlpool" conception of the organism: "La vie est donc un tourbillon plus ou moins rapide, plus ou moins compliqué, dont la direction est constante, et qui entraîne toujours des molécules de mêmes sortes, mais où les molécules individuelles entrent et d'où elles sortent continuellement, de manière que la forme du corps vivant lui est plus essentielle que sa *matière*," (18, p. 13).

"Dans les corps vivans chaque partie a sa composition propre et distincte; aucune de leurs molécules ne reste en place; toutes entrent et sortent successivement: la vie est un tourbillon continuel, dont la direction, toute com-

pliquée qu'elle est, demeure constante, ainsi que l'espèce des molécules qui y sont entraînées, mais non les molécules individuelles elles-mêmes. . . . Ainsi la forme de ces corps leur est plus essentielle que leur matière," etc. (17, pp. 151–152).

Claude Bernard (4) expresses an essentially similar view as follows (p. 51): "Il y a comme un dessin préétabli de chaque être et de chaque organe, en sorte que si, considéré isolément, chaque phénomène de l'économie est tributaire des forces générales de la nature, pris dans ses rapports avec les autres, il révèle un lien spécial, il semble dirigé par quelque guide invisible dans la route qu'il suit et amené dans la place qu'il occupe.

"La plus simple méditation nous fait apercevoir un caractère de premier ordre, un *quid proprium* de l'être vivant dans cette ordonnance préétablie."

Henderson (46) puts the same idea in these words (pp. 23–24): "Living things preserve, or tend to preserve, an ideal form while through them flows a steady stream of energy and matter which is ever changing, yet momentarily molded by life; organized, in short."

The important point in the elementary exposition embodied in our *schema* is expressed in the last clause under B, which is italicized. These seven words are at once a truism and also the statement of one of the most fundamental problems of biology, about which our ignorance is both profound and comprehensively manifold. Perhaps it ought to be stated at this point, so that there may be no misunderstanding, that by pattern or organization is not

meant merely the anatomy of living things. These words are here used to signify the total dynamic functional-structural integration which is the distinctive attribute of an organism. "Pattern" is the term preferred by Whitehead (136); "organization" by L. J. Henderson (47), who follows in this usage the noblest of precedents, that established by Aristotle.

What biology has chiefly studied and is at present studying is the C of our scheme, and the transition from B to C, or in other words the becoming of C. One of the difficulties, perhaps the greatest one of all, which interferes with the direct investigation of the organization of living things is the circumstance that, generally speaking, the very existence of B depends upon the bond between A and B, represented by the arrow in the scheme, being unbroken. If B is to be observed in action A has to be also in the picture, and in practice it is generally impossible clearly to separate that part of a particular vital event which truly is attributable to the pattern of the organism *per se* from that part in which A, and the transitional stages of A through B to C, are also directly involved.

But there are ways in which this difficulty may be avoided. Some years ago there was carried out in my laboratory (89, 105) a series of experiments on the total duration of life of flies (*Drosophila*) in the complete absence of food. To conduct critically an experiment on duration of life under conditions of starvation involves a number of difficulties. For the particular problem here dealt with it was necessary to have a large number of flies

all emerging from pupal to imaginal life at about the same time, and under such conditions that they could not possibly get any food at all as imagoes. To attain this end the flies for these experiments were reared in glass tubes 3 cm. in diameter and 11.5 cm. long. Through the larval and pupal periods the lower end of the tube was closed with a cork stopper on which a layer of standard banana agar of the usual depth had been poured. The top was closed by a cotton plug. At the end of eight days, when the parent flies were removed from the tubes, the bottom cork and attached food were also removed and replaced by a clean cork. During this eight-day period most of the larvae had crawled out of the food and pupated on the sides of the tube, just as in the ordinary breeding bottle. Of course in this way some of the progeny were lost — those which had not pupated or which had pupated on or near the surface of the food — but since all that was desired was to obtain a large sample of flies, raised under uniform conditions, all emerging within a short interval of time and having no food in the adult state, this was no objection to the method. Pupae could, of course, have been individually removed from ordinary breeding bottles for hatching, but this method would have taken much more time, besides introducing the possibility (indeed certainty in many cases) of injuring the pupae in handling. Progeny flies were removed every six hours, etherized, and counted out into clean, empty one ounce vials, in different density groups. Each interval's hatch was distributed proportionately among the various densities.

The next point to be considered was that of moisture. It was obviously undesirable to run the duration of life tests in an absolutely dry atmosphere, because in addition to the starvation there would then be superimposed a desiccation effect due to evaporation of water from the flies' bodies. On the other hand, it was equally undesirable to have standing water where the flies could get at it. To that there are various objections. One important one is that it is practically impossible to furnish water to flies and not have the water contain some trace of nutritive material in solution or suspension, particularly after the first fly has been in contact with it. It should be realized when one is working with very small animals that any experiment which states that drinking water is furnished to otherwise starved individuals, is really not a starvation experiment at all, but an experiment in feeding dilute food, unless the most extraordinary and practically impossible chemical precautions are taken. The theoretically most desirable procedure would seem to be to keep the flies during the experiment in an atmosphere so humid that there would be no significant evaporation from their bodies, and at the same time not sufficiently saturated to precipitate water in the tubes so that the flies could drink it. This state of affairs would prevent any desiccation effect from complicating the results, and also keep the flies from getting minute but real amounts of food in the guise of drinking water. After some preliminary experimentation the conditions desired were realized in the following manner.

All the duration of life bottles were kept in one electric

incubator maintained at 25° C. The atmosphere was kept moist by trays of wet sand placed in the bottom of the incubator. Dry and wet bulb thermometers kept in the incubator throughout indicated that the relative humidity was held practically constant at about 80 per cent. This prevented any significant desiccation of the flies, as indicated by the condition of the dead ones. At the same time no standing drops of water condensed in the tubes.

The dead flies were removed and recorded every six hours, preliminary experiments having shown the total life span to be so short that several observations daily were needed to give enough points to construct life tables.

At this point only the results obtained in one series of bottles in these experiments will be discussed. This is merely to avoid complicating the present line of reasoning with details irrelevant to it. Table 10 gives the data regarding the duration of life, under conditions of starvation, of wild type flies in one ounce bottles in each of which fifty flies were placed at the start (initial density = 50).

If now the final distribution of Table 10 be put upon a relative, instead of an absolute time (age) base, in the manner described in Chapter III, the net result is that the distribution of the differences in inherent vitality between the individual flies in the experiment is mathematically substantially identical with the distribution of corresponding differences in respect of total vitality in normally fed flies. This is clearly shown in Fig. 32.

TABLE X

SURVIVORSHIP DISTRIBUTION AND BIOMETRIC CONSTANTS FOR WILD TYPE DROSOPHILA IN THE COMPLETE ABSENCE OF FOOD

Age in hours	Survivors at indicated age		
	Males	Females	Both sexes together
3	1000	1000	1000
9	997	998	998
15	986	980	983
21	979	973	976
27	976	936	955
33	927	922	925
39	729	837	784
45	319	531	477
51	101	329	216
57	21	149	86
63	0	58	29
69	..	14	7
75	..	3	2
81	..	0	0
Mean duration of life in hours	42.21 ± .31	46.94 ± .44	44.60 ± .28
Standard deviation in hours..	7.69 ± .22	11.08 ± .31	9.85 ± .19
Coefficient of variation	18.23 ± .53	23.61 ± .69	22.08 ± .46
Absolute number of flies.....	288	295	583

Now in terms of the *schema* presented above these starvation life curves were concerned solely with B, whereas similar experiments with normally fed flies involve both A and B and the transition between them. Why it can be asserted that the starvation experiments dealt solely with B, that is, with differences in the pattern or

organization of different individuals, is because whatever nutritive material included in the body, such as cells of

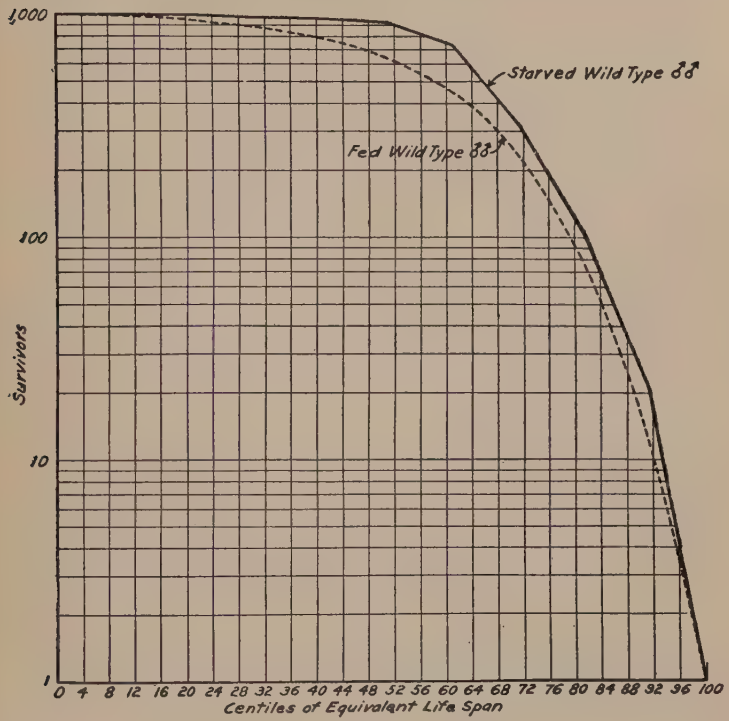

1000
100
10
1
Survivors
Starved Wild Type ♂♂
Fed Wild Type ♂♂
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100
Centiles of Equivalent Life Span

FIG. 32. Comparing the survivorship curves on a centile age basis of, (a) starved wild type males (solid line) and (b) fed wild type males (broken line).

the fat body, muscle tissue, etc., which the flies may have utilized before they died of starvation was, after all,

implicate in and an integral part of the total organization pattern of each individual. The character, availability and every other characteristic of such endogenous nutritive material as may have been used before death by each individual were all part and parcel of that individual's organic pattern, being as they were solely because that particular fly was just that kind of a fly and no other kind.

This being so, and I see no rational possibility of maintaining that it is not, the result which emerged from the experiment seems to have considerable theoretical interest. It shows that, in *Drosophila* at least, the *form* of the distribution of individual differences in *inherent vitality,* meaning that which is dependent solely upon the inherited (i.e., genetically determined) organic pattern of the individual, is the same as that of the distribution of total vitality in which both the organization of the individual and the external environment play a part. With the foregoing experiment as an example of the meaning, I wish to define *inherent vitality,* for the purposes of the following discussion, *as the total potential capacity of an organism to perform vital actions, in the complete absence of matter or energy of exogenous derivation.* From the standpoint of energetics inherent vitality is to be thought of as analogous to the potential energy of a charged Leyden jar.

Another and quite different approach to the direct study of organization can be made through the use of plant seeds as material, taking growth as the index of vitality rather than total duration of life. It is to some experiments (94) along this line, upon which I have been engaged for

some years past, with the able assistance of Miss Agnes L. Allen, that I wish next to draw attention.

The dry seed of a dicotyledonous plant, like a canteloup (*Cucumis melo*) let us say, is a complete but undeveloped individual, containing in the cotyledons, which are morphologically leaves of the preformed plant which the seed includes, stored nutritive material sufficient to carry the seedling on until the nutrition can be obtained by absorption through the roots and by photosynthesis. The cotyledons and the stored nutriment which they contain are an integral part, and a very important part, of the total organic pattern of the individual. If now we sterilize such a seed, plant it on a medium which contains no food material upon which the roots can draw, and keep the whole preparation in the dark, the growth of the etiolated seedling which ensues is an expression of the *inherent vitality* (as defined above) of that individual, since it must draw whatever nutriment it gets from endogenous sources, which are themselves an integral part of the total organized pattern of the individual. Again we shall be able to discuss the B element of our scheme, the organization of the individual, freed from the disturbing influences of A.

There has been, in the literature, some discussion as to whether etiolated seedlings grown entirely in the dark could carry on, through the intermediacy of "etiolin," some slight carbon dioxide assimilation. This view owed its origin to Ewart (24). But the work of Irving (50) would appear to have definitely and finally shown that etiolated shoots have no power of photosynthesis.

Furthermore, Willstätter and Stoll (138) and, more recently, Coward (13) have demonstrated that "etiolin" consists only of the usual carotinoids of leaves, xanthophyll and carotin, and so photosynthetic activity could not be expected of it.

The particular actual experiments to be discussed here, which were a part of a much larger series, were carried out in the following way. Samples of two hundred seeds each were taken from a large population of seeds of the canteloup (*Cucumis melo* var.). These samples were drawn at random except that by selection the total weight of each sample, and therefore the average weight per seed, was made nearly the same.

The seeds of each batch were then soaked in 100 c.c. of sterile distilled water for a period of three hours, in such a way as absolutely to ensure the complete submersion of each seed for exactly the same length of time. The method is shown in detail in Fig. 33.

After the seeds had been soaked for three hours they were removed, drained of adhering water and placed on the surface of an agar plate, as shown in Fig. 34.

The agar plates were poured in Pyrex glass plates 21.5 cm. in diameter. There was poured into each dish 350 c.c. of 1.5 per cent. agar, made with sterilized distilled water, the whole resterilized in the making. The plates after pouring were covered and left over night before sowing the seeds on them, in order for the agar to set in a firm jelly.

After the seeds were distributed on the plates, two hun-

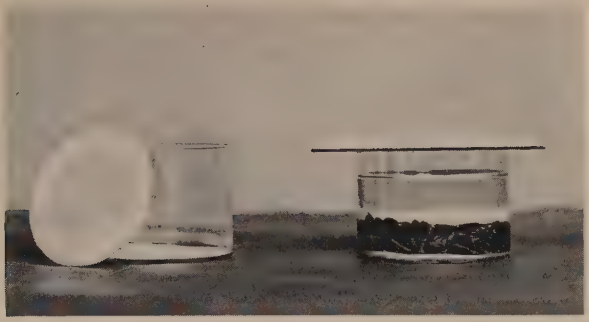


FIG. 33. Showing at left empty dish in which seeds were soaked and cheese cloth ring. The counted seeds are placed on a filter paper slightly larger than the diameter of the dish, and the cheese cloth ring laid over them. Then the filter paper and ring, with seeds between, are carefully thrust down into the dish of water. The appearance of the seeds while soaking is shown on the right hand side where the edge of the filter paper is seen at the bottom, above it the soaking seeds, and above them the cheese cloth piston ring.

FIG. 34. Photograph of 200 seeds on agar plate.

dred to each, and again covered to prevent evaporation and to protect the preparation from contamination, they were placed in an electric incubator running at 30° C. The incubator was completely dark inside.

From this point on the procedure was as follows: The plates were examined at frequent intervals. Whenever any sprouted seeds were observed on a plate they were counted, and then very gently and quickly removed to growing dishes in which 100 c.c. of 1.5 per cent. sterile agar made with sterile distilled water had been previously poured and set. No more than twenty-five sprouted seeds were put in each such growing dish. If more than this number were ready to come off the plate at any given observation time, twenty-five were counted into the first dish, and the remainder, if less than twenty-five in number, put into a second dish, and so on. These growing dishes were then covered and immediately put back into the incubator, and the seedlings in them were allowed to grow in the dark at 30° exactly forty-eight hours. At the end of the forty-eight hour period the dishes were removed from the incubator. Each seedling was then carefully removed from the agar and at once separated with a sharp scalpel into its three morphological components, (a) cotyledons, (b) stem and (c) root. These parts were then put separately into vials of 5 per cent. formalin, in order to preserve them for subsequent weighing. It was necessary to hold them in this way, because the pressure of the experimental work did not permit of doing the fresh weighing at the time.

After an experimental run was finished the material was taken from the vials, all adhering moisture removed by blotting with filter paper, and the parts then weighed to the nearest one tenth of a milligram. The material was then dried at 98° C. and the dry weights of each lot recorded to the nearest one tenth of a milligram.

Reviewing the whole procedure it is seen that what we did was to let the seeds germinate until there was a visible sprout, averaging approximately 1 mm long, keeping exact record of the time it took to do it. These sprouted seedlings were then transferred to a fresh agar surface and allowed to grow exactly forty-eight hours in the dark, and then weighed by parts.

The only important sources of error in the experiment were these: (1) Even with frequent observations there was some variation in the length of the sprouts at the time the seedlings were transferred from the plate to the growing dish to observe the forty-eight-hour growth. This variation was not large, however, and was insignificant in comparison with the total growth obtained. (2) The weights after preservation in formalin were not true fresh weights. In the way the experiment was planned, for quite another object than the present one, it was impossible to take the fresh weights at the time. A special investigation of the point showed that by storage in 5 per cent. formalin there was a loss of 12.7 per cent. in weight in the case of the cotyledons, 10.2 per cent. in case of stems, and 13.6 per cent. in case of roots. Obviously the formalin weights recorded can not be substituted for true fresh weights. But

in the present study, and in the other work for which the experiments were planned, absolute fresh weights were unimportant. The concern was with relative weights of different batches. We have found no evidence that relative weights are altered by the formalin treatment. Furthermore we have found no alteration of dry weights, either absolute, or relative, by this procedure. (3) The agar substrate on which the seedlings grew was a high grade of bacteriological agar, which is of course mainly cellulose. But upon analysis it was found to contain 3.37 per cent. of mineral matter by weight, of which 3.12 per cent. was acid soluble. This means that the whole of the agar jelly in each growing dish contained .00158 gram of acid soluble mineral matter. Assuming that all the acid soluble material was also water soluble and capable of being absorbed by the roots of the seedlings, if the twenty-five plants in a growing dish had taken out *all* of the soluble mineral matter in the dish, each seedling would have got only 0.00006 gram of such matter. But such an assumption greatly overstates the possibility. So I think we may fairly conclude that only a trace of possibly useful mineral matter could have been of exogenous origin in the experiment.

Returning now to the main thread of the discourse, we have provided, by the experimental procedure described, measurements of the initial forty-eight-hours' growth of canteloup seedlings, under conditions controlled and limited with physical precision so that the entire food material for the growth must be derived from endogenous

sources, which are themselves an integral part of the organized pattern of the individual, except for the possibility of a trace of soluble mineral salts or a trace of some nitrogenous compound which may have been brought into the plants through the roots from the agar substrate. This possible exogenous material is insufficient in amount to be in any appreciable degree significant in the results.

What happened in the experiment, of course, was that some seeds germinated earlier than others, and were, in consequence, removed from the sprouting plates to the growing dishes earlier. The record of germination of a typical series is given in Table 11. On account of limitations of time I shall discuss here only this typical Series I. The experiments on which the conclusions reached in this paper have been reached have been repeated and confirmed many times in my laboratory, with a variety of minor modifications in the technique. Here it seems wise to make the discussion of what is at best a complicated matter as clear as possible by confining attention to a single typical series of experimental results.

It is evident that the final percentage of germination attained was high. This is a reflection of the extraordinary usefulness of the agar plate technique. We have tried many other procedures for the accurate study of germination, but have found none to compare with the agar plate method for accuracy, constancy and high absolute germination.

The inherent vitality of a seed, when measured by

TABLE XI

GERMINATION RECORDS. SERIES I

Hours on plate	Seeds germinated	Per cent germinated	Total number germinated	Cumulated per cent germinated
26.1	57	28.5	57	28.5
31.2	48	24.0	105	52.5
43.3	66	33.0	171	85.5
50.7	9	4.5	180	90.0
68.8	3	1.5	183	91.5
95.0	5	2.5	188	94.0
118.6	4	2.0	192	96.0
142.7	3	1.5	195	97.5
166.0	0	0	195	97.5

growth, is clearly a function of two observed elements, namely:

(1) The *amount* of new substance added as the result of the growth, expressible among other ways as the weight, fresh or dry, of the stem and root together (i.e., rest of the plant after removal of the cotyledons).

(2) The *time* the individual took to accomplish the amount of growth specified under 1.

In the present experiments the time during which the seedling was allowed to grow after it had germinated was made constant at 48 hours for each individual. But, as is clearly shown in Table 11, the time taken to reach germination under the uniform and constant environmental conditions of the experiment was quite different for different individual seeds. But this duration interval from the time when the seeds were put on the plate till they

showed visible germination and were transferred to the dishes is clearly a part of the expression of the inherent vitality of the seeds. Those seeds that use a short time in reaching this stage of growth are exhibiting a more rapid rate of action, or a more intense vital activity per unit of time, than those which take a long time to reach the stage of visible sprouting.

The results of the experiments are exhibited in Table 12, which includes the following items:

(1) The absolute time (number of hours) the seeds were on the germinating plate before removal to the growing dishes; that is, before visible germination.

(2) The relative time spent by the seeds on the plates before germination, got by reducing the figures in column 1 to percentages of the total interval up to the time when the last seeds in the lot ever to germinate had done so.

(3) The total number of sprouted-seed-hours exposed to risk of growth. This actuarial expression properly combines into one figure the space time elements which are necessarily and inseparably connected in any quantitative expression of growth. It is got by multiplying the number of hours spent by the sprouted seeds on plate and dish by the number of such seeds.

(4) The absolute weight in grams of the stems and roots together, attained at the end of the 48 hour stay in growing dishes, per 10,000 sprouted-seed-hours exposed to risk of growing. There are two columns of these absolute weights: (a) the formalin " fresh " weights, and (b) the dry weights.

(5) The relative attained weights per 10,000 sprouted-seed-hours, got by transforming the absolute weights of columns 4a and 4b to relative figures, on the basis of taking the mean of the first four observed weights in each series as equal to 1. The reason for making this simple transformation will appear as we proceed.

TABLE XII

GROWTH RECORDS. SERIES I

Hours on plate	Relative time on plate in per cent	Sprouted-seed-hours exposed to risk of growth	Absolute weight of stems and roots in grams per 10,000 sprouted-seed-hours exposed		Relative weight per 10,000 sprouted-seed-hours	
			Formalin "fresh"	Dry	Formalin "fresh"	Dry
1	2	3	4a	4b	5a	5b
26.1	15.7	4233.6	5.526	0.201	1.00	0.99
31.2	18.8	3792.1	6.861	0.256	1.24	1.26
43.3	26.1	6062.3	5.427	0.203	0.98	1.00
50.7	30.6	891.4	4.230	0.154	0.79	0.76
68.8	41.4	350.0	5.117	0.200	1.00	0.98
95.0	57.2	715.6	2.135	0.085	0.39	0.42
118.6	71.5	667.1	1.127	0.062	0.20	0.30
142.7	85.9	572.0	1.163	0.041	0.21	0.20

There are two immediate conclusions of some importance which flow from the experimental data set forth in Table 12.

The first is that, within the limits of the experiments discussed, growth is a self consistent measure of inherent vitality. The seeds which took the longest time in germinating made the least growth in a constant time interval

thereafter. Except for minor fluctuation presumably due to random "errors" in the experimental procedure, this relationship is consistent throughout the table.

The second result is that the measurement of inherent vitality by means of the time rate of growth, as in the experiments here described, leads to substantially the same distribution of differences among individuals in respect of this attribute of the organism that is found if inherent vitality is measured in terms of total duration of life, as in the *Drosophila* experiments under conditions of starvation. This fact is demonstrated in Fig. 35.

In this diagram two lines are plotted. The solid line is the p_x line calculated from the data of Table 10, for starved wild type male flies in bottles of initial density 50. The function

$$p_x = 1 - q_x,$$

gives the probability of living through a period of 6 hours beginning at age x. The abscissae are expressed in Fig. 35 as centiles of the total adult or imaginal life span under conditions of complete starvation, rather than in hours.

The broken line of Fig. 35 is the graph of column 5b of Table 12, plotted against the abscissal points given in column 2 of the same table. The ordinates express the mean relative growth of the canteloup seedlings, per 10,000 sprouted-seed-hours exposed to risk of growing, over a constant period of 48 hours, at the indicated relative times of starting visible growth.

There can be no doubt as to the substantial identity of the two distributions shown in Fig. 35. The seedling curve starts later than the *Drosophila* curve because seedling growth was not measured until it became visible after germination. The seedling curve is irregular in its early

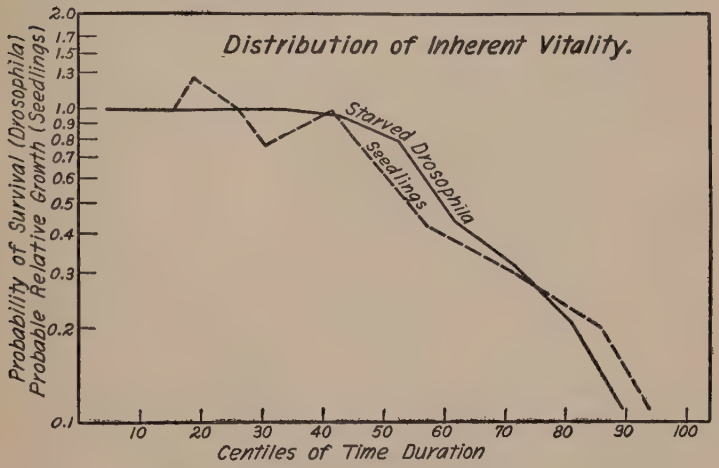


FIG. 35. Comparing the distribution of differences in inherent vitality of *Drosophila*, measured by total duration of life (solid line), with that of the same characteristic in canteloup seedlings, measured by relative growth (dash line), both without exogenous food.

part, because the experiments from which the data here presented were derived were planned for an entirely different purpose from that to which they are here applied. For the original purpose of the experiments it was not essential that all the seeds should come off the plates at precisely the same stage of attained growth. There was

some variation in this matter which reflects itself in the fluctuations at the beginning of the curve.

But the broad fact seems to be demonstrated that differences in inherent vitality as defined in this chapter — the activity of living things which is an expression solely of their innate pattern or organization — have the same type of distribution among individuals, whether this attribute of the organism is measured by total duration of life or by rate of growth. Furthermore, the form of the distribution of differences in inherent vitality among different individuals is substantially the same in such widely different living things as an insect and a cucurbitaceous plant. The experiments on flies and on seeds which furnish the data for the present discussion were made at different times three years apart. In neither case was there any thought of their results being used in support of the ideas upon which the present discussion is based. It is not without a certain impressiveness that two such totally independent pieces of research should, under the influence of a synthesizing idea of much later date, yield such a concordant picture as is shown in Fig. 35.

It is entirely certain that in the experiments here recounted, both those with *Drosophila* and those with seedlings, the observed differences between individuals in respect of vital activity can not possibly be accounted for by differences in the environmental forces acting upon the individuals. They represent, on the contrary, innate differences in the organization of the living individuals themselves. The reason why this assertion can be so positively

made, apart from and beyond the theoretical reasons set forth at the beginning of this paper, is that the environmental conditions surrounding and acting upon each individual were made identical by the most scrupulous exactitude in the experimental procedure. The *possible* differences in the environmental conditions acting upon the different individuals in the experiments were of the order of infinitesimals, in comparison with the large differences in the resulting vital activities of the several individuals.

CHAPTER VII

DURATION OF LIFE AND THE RATE OF LIVING

THE experiments recorded in the last chapter led to two novel conclusions, which wear on their face the aspect of real biological significance. The first of these conclusions was that the distribution of individual differences in *inherent vitality* is of the same form as that of differences in *total vitality*, when vitality is measured by the duration of life of *Drosophila* under conditions of starvation on the one hand, and normal feeding on the other hand, temperature and other environmental conditions being the same in both cases.

But on reflection this conclusion appears paradoxical, because it seems to mean that environmental factors, including food, have no significant effect upon the distribution of the mortality of a group of flies relative to biological age. It suggests that inherent vitality is the basic factor in the determination of the form of a life curve.

What is to be said, however, about the results which were presented in Chapter IV? There it was conclusively shown that density of population not only profoundly affects the absolute duration of life, but also alters the form of the life curve. But density of population is a purely en-

vironmental factor and clearly belongs primarily to the A part of our A–B–C scheme of life processes. Does this factor alter the inherent vitality of the organism? One set of experimental results (those of Chapter IV) indicates that it does; another set (those of Chapter VI) with equal plainness seems to show that it does not. What is the answer?

In general terms the answer is that neither these two sets of experiments, nor any of the others so far described, are sufficient alone to settle the point. This is at once apparent if we schematize the essential elements of the experiments which have been discussed.

I

Experiments on Density (Chapter IV)

Schematic factor = constant $(A + B)$.

Density of population = *variable* { 2 to 500

Other environmental factors = constant.

RESULT: Form of life curve varies with density of population.

II

Experiments on Heredity (Chapter V)

Schematic factor = constant $(A + B)$.
Density of population = constant (optimal).
Other environmental factors = constant

Genetic constitution = *variable* { Vestigial (and other mutant types). / Normal wild type.

RESULT: Form of life curve varies with genetic constitution according to Mendelian laws.

III

Experiments on Inherent Vitality (Chapter VI)

Schematic factor = *variable* { B / and / $A + B$

Density of population = constant (optimal).
Other environmental factors = constant.
Genetic constitution = constant (wild type).
RESULT: Form of life curve same for B and $A + B$ (inherent vitality and total vitality).

This schematic exposition of the state of the case makes it clear that some more experiments are needed to knit together those that have been described. These are:

IV

Schematic factor = constant B (organization pattern only).

Density of population = *variable* { 5 / 50 / 100

Other environmental factors = constant.

Genetic constitution = *variable* { Vestigial / Normal wild type.

The results of the experiments under plan IV will be found described in complete detail in (105). Here only a

brief résumé is necessary. The experiments were done under conditions of complete starvation. This was to satisfy the first condition, namely that we should deal only with the B element of the A–B–C scheme, namely organization pattern. The details of the experimental procedure to ensure this need not be repeated here, as they have already been set forth fully in Chapter V.

The results are shown in Table 13.

TABLE XIII

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF STARVED FLIES. SEXES SEPARATE

Density (flies per bottle)	Mean (hours)		Standard deviation (hrs.)		Coefficient of Variation	
	♂	♀	♂	♀	♂	♀
	Wild type. Line 107					
5	44.50 ± .36	45.03 ± .36	8.77 ± .25	8.91 ± .25	19.70 ± .59	19.79 ± .59
50	42.21 ± .31	46.94 ± .44	7.69 ± .22	11.08 ± .31	18.23 ± .53	23.61 ± .69
100	44.30 ± .29	50.89 ± .40	7.64 ± .20	10.73 ± .29	17.24 ± .47	21.09 ± .59
	Vestigial					
5	43.97 ± .33	48.72 ± .45	8.24 ± .23	11.31 ± .32	18.74 ± .55	23.22 ± .68
50	44.07 ± .35	50.31 ± .47	8.95 ± .24	12.12 ± .33	20.31 ± .58	24.09 ± .69
100	44.74 ± .27	52.47 ± .37	7.30 ± .19	9.98 ± .26	16.31 ± .43	19.03 ± .52

SEXES COMBINED

	Mean (hours)		Standard deviation (hrs.)		Coefficient of Variation	
	Wild type	Vestigial	Wild type	Vestigial	Wild type	Vestigial
5	44.77 ± .25	46.36 ± .29	8.84 ± .18	10.19 ± .20	19.76 ± .42	21.97 ± .46
50	44.60 ± .28	47.20 ± .30	9.85 ± .19	11.11 ± .21	22.08 ± .46	23.53 ± .48
100	47.59 ± .26	48.51 ± .25	9.88 ± .19	9.53 ± .18	20.75 ± .41	19.64 ± .38

The survivorship distributions, for both sexes together, are shown in Fig. 36.

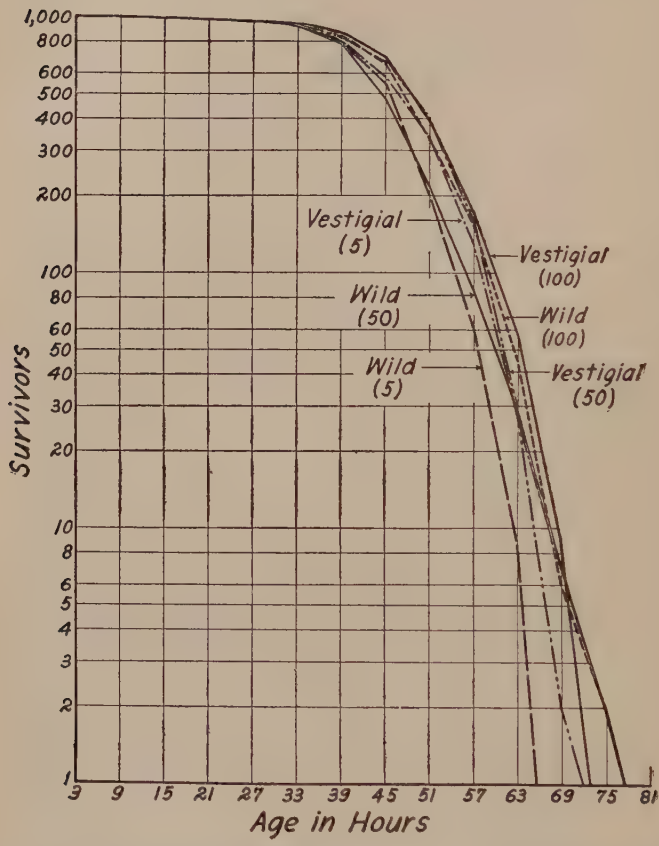


FIG. 36. Survivorship distributions for normal wild type and vestigial *Drosophila*, under complete starvation at three densities of population.

The net result of these experiments is evident from the diagram. Under conditions of complete starvation, in which we are dealing with inherent vitality solely, different densities of population over the range from 5 to 100 inclusive, produce no significant effect whatever upon duration of life. The mean duration of life in hours is approximately the same, about 44 hours, for male flies and roughly 10 per cent higher for female flies, at all densities and for both wild type and vestigial flies.

This result I take to mean the following things:

1. That the *inherent* vitality of an individual fly is not, in fact, altered by the environmental circumstances in which its life is lived. It is, on the contrary, of the nature of a constant for the individual, in the sense that the morphology of a leg, for example, is constant for the individual.

2. That the difference between normal wild type flies and vestigial flies in respect of duration of life, which under normal conditions of feeding (that is when it is the expression of the total vitality implicit in the normal A + B physiological economy) follows the Mendelian laws of inheritance, is *not* dependent upon a fundamental difference in *inherent* vitality. This difference, on the contrary, appears merely to be due to the fact that under the environmental conditions represented by the standard fly husbandry of the laboratory (the A of our schema) vestigial flies were not able to bring their inherent vitality to

so complete expression in duration of life as were the wild type flies under the same conditions.

This case is a specific example of the general principle that the somatic expression of any genetic factor in any particular case is in part a function of the general environmental level which prevails in that case. It has been demonstrated, as already stated, that *under the standard feeding conditions for laboratory bred Drosophila* the gene for vestigial has as a part of its somatic expression a very considerably reduced duration of life as compared with the wild type. There are few cleaner-cut cases of Mendelian segregation to be found in the whole literature of genetics than that upon which the above statement is made. Yet the results just described show that the whole of that part of the somatic expression of the vestigial gene which is differential in respect of duration of life disappears under another system of " feeding " wild type and vestigial flies (namely, complete starvation). This fact does not in the least invalidate the earlier results cited on the inheritance of duration of life. Those results are *facts* just as much as the observations under starvation.

Jennings (51) has stated with great clearness the general biological principle which operates here, in discussing his results on the genetics of unicellular organisms. He says (pp. 84–86): " An organism's heredity is its method of responding to the environmental conditions. Under a given environment the genotype *A* is large, while the genotype *B* is small. Under a given environment the strain *C* conju-

gates, while *D* does not. Under a given environment the strain *E* divides rapidly, *F* slowly or not at all. The various strains thus differ hereditarily in these respects, and we may say that the differences are matters of heredity. And yet we can get these same contrasts within any genotype, by varying the environment. The genotype *A* under one environment is large; under another it is small. Under one environment the type *C* conjugates; under another it does not. Under one environment *E* divides rapidly; under another, slowly. Are then size, conjugation and rate of fission after all determined by heredity or by environment? Such a question, when thus put in general terms, is everywhere an idle and unanswerable one. All environmental effects are matters of heredity when we compare types differing in their reaction to the environment; all hereditary characters are matters of environmental action when we compare individuals of the same heredity under effectively different environmental conditions. Heredity has a meaning only when we (explicitly or implicitly) compare two concrete cases; when we say: To what is due the *difference* between these two cases? Otherwise we can demonstrate either that all characteristics are hereditary; or, with Brooks, that there is no such thing as heredity. If we always compare two concrete cases, asking to what is due the difference between them, and remember that a difference in heredity means different response to the same environment, we shall avoid these confusions, and shall find the concept of heredity most useful."

The second of the conclusions about inherent vitality reached in the preceding chapter was that the distribution of individual differences in inherent vitality is of the same form when measured as the integrated time variable total duration of life, or as the differential time variable, rate of growth.

This conclusion is based upon the comparison of the distribution of individual differences in mortality of one organism (*Drosophila*) with the distribution of individual differences in growth rate of another widely different organism (*Cucumis melo*), the experiments being in both cases so planned as to give an expression solely of inherent vitality. But plainly this second conclusion cannot be regarded as completely established until it has been shown that either duration of life or rate of growth gives a consistent index of inherent vitality, within the limits of experimental error, *in the same individual organism.*

Experiments on this point may most conveniently be carried out with seedlings as material. We have performed several series of them with canteloup seedlings. They confirm and extend the conclusion which has been stated above. A typical series may first be described in some detail, followed by a more condensed account of others.

The specific problem, which has been determinative of the particular plan according to which these experiments have been set up, may be put in this way. What is the relation between growth rate and duration of life in a group of seedlings when maintained in a uniform environment constant as to temperature, absence of light and food,

amount of moisture, orientation, etc., so chosen that all shall have the same inherent vitality, as nearly as possible? In the last clause of the formulation of the problem a working postulate is implied. This postulate is that if seeds are of identical genetic constitution and size (mass) they will each have the same inherent vitality, to a first degree of approximation. At the present stage of the discussion this postulate is, of course, nearly pure hypothesis.

Glass tubes, closed at one end like a test tube, are made by the glass blower. These tubes have the following dimensions; length 60 cm., inside diameter, 20 mm. In each of these tubes is poured 40 c.c. of a 2.5 per cent solution of agar in distilled water. The tubes and their contents are then sterilized in an autoclave. After the agar has cooled and set to a jelly there is placed on its surface, with aseptic precautions, one seed of *Cucumis melo,* which has been prepared in the following manner. In the first place all the seeds for a given experiment are the produce of a single melon grown the year before in our experimental garden. In this way approximate genetic homogeneity in the experiment is secured. The seeds to be used are selected for uniformity of size. Each seed is individually weighed and measured and the selection for uniformity based upon these quantitative data. Then, under aseptic precautions, the testa is removed from each selected seed (95). The shelled seeds are then sterilized by immersion for one minute, with stirring, in a 1:1000 bichloride of mercury solution, followed by rinsing in sterile distilled water. The seeds are then soaked for 3 hours in sterile, distilled water,

in such a way as to ensure that each seed is not in contact with any other, and that therefore all have an equal chance to absorb water. After soaking, one seed is carefully placed on the top of the agar in each tube. The tube is then tightly sealed with a sterile cork or with sterile cotton, which is never thereafter removed until the experiment is ended. The tubes are placed in light-tight boxes and put in an electric incubator running at 30° C.

Each day thereafter the tubes are examined in a dark room, illuminated only by a photographically non-active ruby light. The height of the stem is measured in millimeters, by applying a scale to the side of the glass tube.

What happens in such an experiment falls into the following periods:

a. The period of growth. During this period the seedling is actively growing. A richly branched root system develops in the agar substrate. Above it rises a straight unbranched stem, bearing the cotyledons at its top. The growth of the stem in length follows a logistic curve of the type discussed by Pearl and Reed (90, 91, 92, 108–113) and Yule (141). The seedling growth curves are slightly asymmetrical (cf. 113), but to a first approximation are sufficiently well graduated by the simple logistic,

$$y = \frac{K}{1 + e^{a + bx}}.$$

b. The period of suspended animation. After growth has ended the seedling remains without visible change for a varying number of days, not growing but still living,

with cells in full turgor, and in every way entirely normal in appearance.

c. The period of death. Finally a time comes when the seedling begins to die. Death is a progressive process which requires a number of days to complete. The process may visibly begin in either one of several different ways. But with few exceptions all the seedlings die in one or the other of two characteristic ways. These we have come to designate in the laboratory as respectively " wet " deaths or " dry " deaths. In the former the first evidence that the seedling is beginning to die is the appearance of fluid in fairly large drops on the surface of the stem. Following this the stem gradually gets translucent, losing the ivory white opacity which characterizes its appearance in full vigor. It finally becomes limp and watery along its entire length, and ultimately collapses to the bottom of the tube. The whole appearance in the " wet " deaths is of a gradual but continuous sterile autolysis of the stem.

In the " dry " deaths the first appearance in the series of changes leading continuously to complete death is of a shrinking of some portion of the stem in diameter but without the appearance of fluid on its surface. The shrunken area gradually extends along the stem. Finally just a day or two before the complete collapse fluid does appear on the stem just as in the " wet " deaths, and the end is general autolysis as in the other cases.

The gradual progressive nature of the death of the seedling makes a practical trouble in the experiments. It is

difficult to decide upon and to read an end-point of total duration of life. The series of events described above are nowhere sharply and precisely delimited. The stages grade into each other by a gradual continuous process difficult to break up observationally into discontinuous phases, for the simple reason that it *is* fundamentally and inherently continuous.

We have studied various " end-points," and have found the most reliable and least variable, because capable of being read with the greatest certainty, to be the beginning of death (A), as evidenced by the first appearance of abnormality of the stem, in the continuous series which we have learned invariably ends finally in the complete death of the seedling.

In the first series of experiments to be described the tubes numbered 55 at the start. One of the seeds (Tube 42) failed to germinate. In two others (Tubes 29 and 35) the germination was abnormal and resulted in malformed seedlings. One tube (No. 40) was accidentally broken in handling during the experiment. This left 51 normal seedlings. Five of these had to be discarded subsequently because they turned out to have been contaminated in the course of the initial manipulations, and later showed growth of bacteria and mould. This left 46 tubes which completed the definitive experimental run. The essential numerical data regarding them are given in Table 14. The figures given in the column headed " Growth rate constant *b* " are the values of the rate constant *b* of the logistic curve.

$$y = \frac{K}{1 + e^{a + bx}}.$$

which was fitted to the observed records of linear growth of the stem in each tube.

TABLE XIV

GROWTH AND DURATION OF LIFE OF SEEDLINGS OF CUCUMIS MELO GROWN IN TOTAL DARKNESS AT 30° C. WITHOUT EXOGENOUS FOOD

Tube No.	Duration of period of growth Days	Total stem length (L) at end of growth Cm,	Growth rate constant b.	Duration of life Days A.	Tube No.	Duration of period of growth Days	Total stem length (L) at end of growth Cm.	Growth rate constant b.	Duration of life Days A.
1	15	20.7	0.725	32	25	13	24.1	0.794	23
2	15	23.7	.774	30	26	11	24.5	.861	25
3	15	23.1	.806	29	27	11	20.9	1.027	20
5	13	22.2	.829	28	28	14	24.2	.809	27
6	13	25.0	.794	26	30	15	24.0	.747	30
7	15	24.0	.795	26	31	22	24.9	.723	29
8	14	23.0	.843	28	32	15	25.0	.956	29
9	14	23.5	.798	27	33	11	22.2	.889	21
10	14	21.7	.799	27	34	17	24.3	.835	28
11	13	21.5	.854	29	36	13	24.8	.818	29
12	16	23.7	.828	28	37	13	24.2	.782	27
13	13	23.4	.799	29	38	13	23.7	.767	28
14	15	23.6	.729	29	43	16	21.8	.779	28
15	15	27.3	.705	29	44	13	24.0	.784	27
16	11	24.0	.803	27	46	14	22.9	.769	27
17	16	25.4	.747	27	48	11	25.5	.725	24
18	13	24.3	.805	29	49	15	23.1	.728	28
19	14	21.9	.741	28	50	14	22.0	.791	30
20	14	24.2	.815	29	51	14	24.6	.765	27
21	14	23.2	.761	27	52	22	28.0	.747	28
22	16	22.5	.810	29	53	13	22.6	.807	25
23	15	25.0	.805	27	54	11	24.3	.816	25
24	13	24.1	.794	27	55	13	25.6	.744	28

From the data of Table 14 the biometric constants shown in Table 15 are derived by the usual method, without grouping:

From these data it is evident, in the first place, that the period of growth, and the period of " suspended animation " after growth is completed and before death sets in,

TABLE XV

BIOMETRIC CONSTANTS FOR GROWTH AND DURATION OF LIFE OF SEEDLING OF CUCUMIS MELO

Character	Mean	Standard Deviation	Coefficient of Variation
Duration of growth period (G) in days.....	14.13 ± 0.22	2.23 ± 0.16	15.80 ± 1.14
Logistic growth rate constant (b).....	0.7962 ± 0.0057	0.0578 ± 0.0041	7.26 ± 0.51
Total stem length in cm.	23.74 ± 0.15	1.46 ± 0.10	6.14 ± 0.43
Total time to beginning of death (A) in days.	27.39 ± 0.22	2.20 ± 0.15	8.04 ± 0.57
Original weight of dry seed (S) in mg.	13.44 ± 0.06	0.63 ± 0.05	4.71 ± 0.33

were in this series substantially equal in duration. The average period of growth is 14 days, while that of "suspended animation" is $27.39 - 14.13 = 13.26$ days.

In the second place, the growth of the seedling, whether measured by the total attained length of the seedling at the end of the growth period, or by the rate constant of the logistic curve, is a much less variable phenomenon than is the time used in making this growth.

The least variable of all the characters discussed is the initial weight of the dry seed. These were selected to relatively great uniformity, as has already been explained.

The important variables here are (G), the duration of the growth period, (b) the growth rate constant of the logistic curve, (A) the total time to the beginning of death (duration of life), and (S) the initial weight of the dry

seed with testa removed (net weight of dry embryo including cotyledons). By an analysis of the inter-correlations of these variables light is thrown upon a number of problems regarding the biology of death and life duration.

There is, as would be expected, a significant negative correlation between the length of the growth period, and the rate of growth.

$$r_{Gb} = -0.362 \pm .086.$$

The longer the duration of the period of growth the smaller is the rate of growth per unit of time. This result would be expected for purely numerical reasons, considering the small variation in the total amount of growth achieved, which has been shown above.

Again largely for purely numerical reasons, there is a significant positive correlation between the duration of the growth period and the total time to the beginning of death.

$$r_{GA} = +0.476 \pm .077.$$

The longer the duration of the growth period the greater is the total duration of life to the beginning of death. The growth period, as we have seen makes up almost exactly a half of the total time to the beginning of death on the average.

Between the initial weight of the dry seed (with testa removed) and the duration of the growth period, on the one hand, and the total duration of life to the beginning of death, on the other hand, there are, in both cases, small and statistically insignificant positive correlations.

$$r_{SG} = +0.199 \pm .095,$$
$$r_{SA} = +0.106 \pm .098.$$

These results mean that there is no significant association between the initial weight of the seed and the time it takes for the completion of growth, or the total duration of life to the beginning of death. But, on the other hand, there is a significant negative correlation between the initial dry weight of the seed and the time *rate* of growth.

$$r_{Sb} = -0.331 \pm .089.$$

These results are biologically interesting. One measureable physical attribute of that organic system which we call a canteloup seedling is its mass. But it appears this attribute is not significantly related to the absolute time variables which define or delimit the vital actions of the system. It is, however, significantly correlated with the percentage first derivative of these vital actions relative to time, namely the *rate* of growth. One might suppose *a priori* that since the bulk of the weight of the dry seed (without testa) is the weight of the cotyledons, and since it is the cotyledons which furnish the energy and material (other than water) upon which the growth and continuation of life must depend under the conditions of these experiments, that there would be a significant positive correlation between the mass of the system and the absolute time duration of growth and of life. The heavier the cotyledons the more food supposedly would be available, whence it might further be supposed that growth and life duration would be able to go on longer. What the actual experimental results indicate is that, within the narrow

range of variability of initial seed weight here included, differences in the *organization* of the system are much more important than small differences in mass in determining the course of vital events.

Between growth rate and duration of life to the beginning of death the correlation is negative and significant in degree.

$$r_{bA} = -0.463 \pm .078.$$

This result is of interest in two directions. In the first place this coefficient, which is 5.9 times its probable error, demonstrates that there is a significant association between individual differences in rate of growth on the one hand, and duration of life, on the other hand, in this group of seedlings, grown under such conditions that both of these phenomena are necessarily dependent upon the inherent vitality of the individual as here defined.

There can be no reasonable doubt as to the correctness of the conclusion that consistent and accordant results will be obtained if inherent vitality, the manifestations of which are dynamic expressions of the organization, be measured by observation either of growth or of duration of life. This result, in short, confirms the second conclusion reached in the preceding chapter.

In the second place the negative sign of this coefficient gives definite experimental support to the general theory of the biology of life duration expressed in the *Biology of Death* (87). The suggestion was there made that duration of life is a function of two variables.

a. The constitution (organization or pattern) of the individuals, genetically determined.

b. The average rate of metabolism or rate of energy expenditure during life.

On this view if the average rate of metabolism or of energy expenditure is high the duration of life will tend to be shortened, while if, for any reason, the average metabolic rate or of energy expenditure is low the total duration of life will tend to be prolonged.

Since the publication of the *Biology of Death* two lines of new evidence supporting this view have been brought forward. The first was from a detailed statistical study of human mortality in relation to the amount and intensity of physical labor (91). In that study it was shown that "taking as a basis of analysis what may be fairly regarded as the most comprehensive and accurate statistics of occupational mortality now in existence, it appears that in occupied English males, there is a direct and positive relation between the magnitude of the age specific death rates from 40 to 45 on, and the average expenditure of physical energy in occupation, after accidental deaths, and deaths directly resulting from the hazards of each of the several occupations, have been deducted in so far as the official statistics by causes of death permit such deductions. This relation is of the sort that associates high mortality with hard physical labor. This relationship prevails whether the labor is performed chiefly indoors or chiefly outdoors. It is not primarily to be attributed to the general environmental factors connoted by social class

distinctions, which are themselves correlated with average energy expenditure in occupation."

The second line of evidence is that presented in this chapter, where the *negative* correlation between growth rate and duration of life shows that the faster the seedling grows (that is the higher the rate of energy expenditure in growing) the shorter its duration of life, and *vice-versa*.

This negative correlation is not sensibly altered if the original dry weight of the seed, or the total attained growth of stem (L), are held constant, separately or together, as the following partial correlation coefficients show:

$$r_{bA.S} = -0.456 \pm .079,$$
$$r_{bA.L} = -0.462 \pm .078,$$
$$r_{bA.SL} = -0.456 \pm .079.$$

These partial correlation coefficients again support the conclusion that it is the organization and not the mass of the system which is biologically more important, at least within the narrow range of variation in initial weight of the seeds here discussed.

With wider experience and consequently increased knowledge it has become possible to observe more precisely and consistently the precise point of the beginning of death in these seedling experiments. When this is done the correlation between growth rate and total duration of life gives still higher numerical values.

In the latest series of experiments, involving 46 tubes, we find the correlation between growth rate and total duration of life to be

$$r = -0.643 \pm .057.$$

This coefficient is more than 11 times its probable error.

These results make it possible to write a regression equation from which the total duration of life of a canteloup seedling to the beginning of death can be predicted from a knowledge of its rate of growth alone, under experimental conditions such that inherent vitality alone is involved.

Thus we have from this last experimental series:

Duration of life (in days) = 35.02 — 8.115 Growth rate (in cm. of stem length per day).

The standard deviation of an array from this equation is 1.96 days. *In short if the growth rate of a canteloup seedling under the conditions of these experiments is known, its probable total duration of life can be predicted with probable error of about 1⅓ days.*

It is desirable now, with the present results in hand, to discuss somewhat more in detail the concept of inherent vitality. In originally defining "total vitality" (94) stress was laid upon the dynamic aspect of its manifestations, and the definition of inherent vitality was merely derivative from that of total vitality. We are in position now to define more comprehensively and precisely inherent vitality.

Neither growth nor total duration of life as observed in the experiments here recounted can be regarded as measuring the absolute value or magnitude of the total inherent vitality of a canteloup seed, under the stated experimental conditions. Each of these phenomena is only the dynamic expression of a part of the inherent vitality. Obviously the

total potentiality of the inherent vitality is not used up in the growth of the seedling, because after the growth is completed the plant remains alive for a period of time substantially equal to that during which it was growing. There being no exogenous sources of matter or energy under the experimental conditions, plainly the ability to keep on living after the end of the growth period depended upon the utilization of the matter and energy still available as an integral part of the original system.

If growth alone cannot be regarded as furnishing a measure of the total inherent vitality of the seed, neither can total duration of life alone. For we have shown that the observed magnitudes of this variable stand in significant association inversely with the rate at which growth has proceeded.

These considerations suggest that the rational expression for inherent vitality is of the general form

$$V - k_1 m_1 v_1^2 - k_2 m_2 v_2^2 - \ldots - k_n m_n v_n^2 = 0,$$

where V is inherent vitality, and quantities like $k_1 m_1 v_1^2$ denote energy expended in particular vital actions such as growth, basal metabolism, etc. The amounts of these separate energy expenditures are determined by the mass of the system undergoing the particular specified vital action, and the velocity with which it occurs. The k's are constants relating different vital actions to each other in respect of their energetics.

There is no theoretical difficulty in determining experimentally the value of V for a particular organism to at

least a first degree of approximation. It only requires that first the more important vital actions such as growth, basal metabolism, mechanical work, and the like, be separately observed, and their energy consumptions be measured. In the case of the seedling experiments here described the measurements necessary for the calculation have not been made. It is idle to attempt the calculation by indirect methods since *ad hoc* experiments can be made, and will be.

But even the indirect data here available permit certain definite inferences regarding inherent vitality.

These are:

1. Inherent vitality is clearly to be thought of as a capacity rather than as a velocity or rate. It may be taken to mean the *total potential capacity of an organism to perform vital actions, in the complete absence of exogenous derivation of matter or energy*. From the standpoint of energetics solely it is analogous to the potential energy of a charged Leyden jar.

2. The behavior of inherent vitality relative to time is that the more rapid the rate of its utilization in the performance of vital actions the shorter will be the total time during which these actions continue (duration of life). This conclusion is the same in principle as that reached by Murray (74) from a different line of evidence. He says (p. 618): "It is as if the catabolic activity (or function) of the living protoplasm was the exciting cause or furnished conditions for certain chemical changes (differentiation of internal form) which in turn lead to a decrease

in catabolic rate. If 'aliveness' is measured by the velocity of chemical activity (heat production) and organism may in this sense be said to dig its own grave. The more abundant its manifestations of life the greater will be its rate of senescence."

3. Inherent vitality is a function of the organization of the individual, which so far as is now known is determined by inheritance.

The general conclusions to which the results so far obtained seem to point as suggestions for further research are these:

First, that the organization or pattern of the living organism has as one of its implications an inherent vitality which is the total potential capacity of the organism to perform vital actions in the complete absence of matter or energy of exogenous derivation.

Second, that the expression of inherent vitality in vital actions may be measured either by total duration of life or by rate of growth, with quantitatively consistent results.

Third, that the relation between rate of growth and duration of life in the same individual is an inverse one, so that the sign of the significant correlation between the two manifestations of inherent vitality is negative. *In general the duration of life varies inversely as the rate of energy expenditure during life.*

CHAPTER VIII

SUMMARY

In this book I have endeavored to present in the briefest possible manner, the results of a series of experimental investigations regarding the general biology of mortality and duration of life which have extended over a number of years. However succinctly told the story is a long one and its plot is involved. It therefore is desirable at the end to bring together into one condensed statement the outstanding results, so that whatever broad significance and consistency they may have will be apparent as a whole.

We start with a single organism, the fly *Drosophila melanogaster* and its mutant vestigial. Under standard normal conditions life tables are constructed for populations of these flies. It is found that the life curves so obtained for the normal wild *Drosophila* are nearly identical to those for human beings, when relative age is used as the yardstick. The life duration of these flies in days is almost identical with that of man in years. But the life curves for the vestigial mutant are quite different. The absolute duration of life of the vestigial flies is only about a third to a half as long as that of the wild type flies. Furthermore the form of the life curve is quite different.

This difference in the life curve of the two kinds of flies is shown experimentally not to be exclusively determined by environmental influences, because it exists and is fully expressed when both kinds are reared under precisely the same normal environmental conditions. Under such conditions the short duration of life and form of life curve characteristic for vestigial flies behaves exactly like a simple Mendelian character when vestigial and wild type flies are bred together.

At the same time one particular environmental factor, density of population, is capable of converting the normal life curve characteristic of the wild type flies into the diagonal type of life curve, with its short absolute duration of life characteristic of vestigial flies at optimal densities of population.

These results make it necessary to attempt to get deeper into the matter by going back to biological fundamentals. From the standpoint of energy transformations the phenomena exhibited by living organisms may be represented schematically as shown at top of next page.

As an implicit consequence of its pattern or organization the organism possesses *inherent vitality,* which is demonstrated by the observable fact that organisms are able to perform vital actions in the absence of exogenous sources of matter and energy. As a working hypothesis inherent vitality is defined as the total capacity of the organism to perform vital actions in the complete absence of matter or energy of exogenous derivation.

This conception makes it possible to proceed along new

A ⟶	B ⟶	C
External Sources of Energy and Non-Living Matter	*The Organism*	*The Product*
(Food. Either chemical compounds of external origin which yield energy and utilizable materials when split by the organism (animals), or similar compounds synthesized by the organism itself, with energy derived from light, and subsequently split to yield energy and utilizable material.)	(An aggregation of matter which has the property of autonomously changing A to C, *by virtue of its pattern or organization.*)	(Either (a) heat, (b) work, (c) component matter of the organism, or (d) waste, meaning chemical compounds incapable of yielding energy with economic profit by further splitting.)

lines in the investigation of the biology of life duration. When wild type flies are reared under conditions of complete starvation (no matter or energy of exogenous derivation) and life curves are calculated from the mortality under these conditions it is found that these life curves are identical in form, on a relative age base, with those for the same kind of flies (wild type) when fully fed, and so deriving their matter and energy through food from exogenous sources. Thus the *form* of the life curve is the same whether the duration of life of the individuals composing the group is an expression of total vitality or only of inherent vitality.

Under the same conditions of complete starvation differences in density of population produce no effect on the duration of life or the form of the life curve of either wild type or vestigial flies. This means that the profound effect of density of population upon the life duration of normally fed flies is not the result of any alteration of the inherent vitality of such flies, but merely curtails the bringing to expression of the potentialities of the inherent vitality. This principle, which is fundamental for all genetics, is furthermore strikingly exemplified in the fact that, under conditions of complete starvation, the life curve of vestigial flies becomes substantially identical in form and duration with that of wild type flies. This means, on the hypothesis developed in this book, that vestigial and wild type flies both have approximately the same inherent vitality, but that under food conditions which are normal and presumably nearly optimal for wild type flies, the potential capacity of vestigials in inherent vitality is able to come to only about one-third to one-half its complete expression. An analogy will perhaps make this point clearer. Suppose two brothers to be equally capable as distance runners, as evidenced by equal performance when both are nourished on a standard diet in which the protein is derived chiefly from beefsteak. Suppose now that one of the brothers has a marked allergic idiosyncracy to lobster protein, and the other does not. Now let them go, for the purpose of competition in races, to a country in which the only diet they can get carried its protein chiefly in the form of lobsters. They would still be

runners of potentially equal capability, but one would be totally incapable of expressing his potential capacity in that environment. He would be in a physiological state which would make fast running impossible.

By the definition of inherent vitality it is evident that duration of life is by no means its only expression. Growth is obviously another. But if inherent vitality is a potential capacity for energy expenditure, implicit in the organism by virtue of its organization pattern, it is reasonable to expect that differences among individuals in respect of inherent vitality would have the same form of distribution regardless, within wide limits, of the particular vital action measured. Experiments with duration of life of *Drosophila* and with growth of canteloup seedlings, both under conditions such that these vital actions are the expression solely of inherent vitality, show that in fact this is so. The distribution of individual differences of duration of life in the former case and of growth rate in the latter case, under these conditions, turns out to be the same in form.

If rate of growth and duration of life be measured for the same organism, canteloup seedlings, under conditions such that only inherent vitality is expressed, it appears that there is a substantial negative correlation between these two variables. The faster the individual grows the shorter its duration of life.

All of the evidence presented in this book converges to the conclusion that, *in general,* the duration of life varies inversely as the rate of energy expenditure during its con-

tinuance. *In short, the length of life depends inversely on the rate of living.*

These results and conclusions are to be regarded merely as stages in a continuing program of experimental research, which I hope will be carried much farther in my laboratory, and certainly will be by the great brotherhood of working biologists generally. I am the last person in the world to hold these conclusions to be at this stage final and incapable of change. The discovery of the laws of nature is a long and tedious process. It progresses chiefly by the experimental testing of hypotheses. This book is merely a modest contribution in this direction. The ideas it suggests are capable of application and testing in many other directions besides those discussed here, notably in the field of human biology, but I prefer to leave these matters for treatment at some future time.

APPENDICES

I.

LIFE TABLES FOR DROSOPHILA

THE actual observations on which these life tables are based include a total of 2,822 wild type flies of line 107, and 980 pure vestigial flies. The observations are recorded in Table 16 on a 6 day base unit in the case of wild type flies, and a 3 day base unit in the case of vestigials. We have found these units sufficiently fine for purposes of tabulation and derivative computation. The actual observations were made daily; the data as presented in Table 16 have been subsequently grouped.

TABLE XVI

OBSERVED DEATHS (d'_x) AND SURVIVORSHIP (l'_x) IN WILD TYPE LINE 107, AND PURE VESTIGIAL FLIES

Age in days	Wild type, Line 107				Age in days	Pure Vestigial			
	Males		Females			Males		Females	
	d'_x	l'_x	d'_x	l'_x		d'_x	l'_x	d'_x	l'_x
1	18	1000	23	1000	1	1	1000	11	1000
7	12	987	16	984	4	48	998	43	979
13	26	979	34	972	7	81	893	43	897
19	85	960	86	941	10	86	715	57	815
25	99	900	86	888	13	81	526	55	706
31	132	829	96	827	16	51	349	63	601
37	187	736	159	759	19	33	237	47	481
43	173	603	152	647	22	35	164	51	391
49	206	480	162	539	25	22	88	27	294
55	285	333	275	425	28	5	39	35	242
61	82	131	156	230	31	4	29	30	176
67	62	72	99	120	34	3	20	17	118
73	36	28	64	50	37	3	13	14	86
79	3	3	6	5	40	2	7	14	59
85	1	1	1	1	43	1	2	4	32
—	—	—	—	—	46	—	—	6	25
—	—	—	—	—	49	—	—	4	13
—	—	—	—	—	52	—	—	2	6
—	—	—	—	—	55	—	—	1	2
Totals	1407	—	1415	—	Totals	456	—	524	—

A good deal of study has been given, at one time and another, in my laboratory to the problem of graduating fly life curves. The type of curve finally found to be most suitable, from a practical point of view was;

$$\log l_x = e^{\alpha x}(a + bx + cx^2 + dx^3).$$

This amounts to asserting that the instantaneous death rate increases with age as a modified logarithmic function of x.

The actual equations used for the present life tables are as follows:

Wild Type, Line 107—Males;

$$\log l_x = e^{.02252584x}(2.9999414 - .0674377x + .000677752x^2 - .0000036932 1x^3).$$

Wild Type, Line 107—Females;

$$\log l_x = e^{.02541248x}(3.0000256 - .0761521x + .0000854457x^2 - .0000044970 5x^3).$$

Vestigial—Males;

$$\log l_x = e^{.06729445x}(2.9961959 - .1959626x + .0045641 5x^2 - .0000377354x^3).$$

Vestigial—Females;

$$\log l_x = e^{.03916697x}(3.0019283 - .1188025x + .0016531 1x^2 - .0000084281 6x^3).$$

TABLE XVII

LIFE TABLE FOR DROSOPHILA — WILD TYPE. LINE 107 — MALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.2	45.8	31	825	16.1	21.1
2	1000	0.6	44.8	32	811	17.2	20.5
3	999	1.0	43.8	33	798	18.3	19.8
4	998	1.3	42.9	34	783	19.5	19.1
5	997	1.7	41.9	35	768	20.8	18.5
6	995	2.0	41.0	36	752	22.3	17.9
7	993	2.4	40.1	37	735	23.8	17.3
8	991	2.8	39.2	38	717	25.4	16.7
9	988	3.2	38.3	39	699	27.2	16.1
10	985	3.5	37.4	40	680	29.1	15.5
11	981	3.9	36.5	41	660	31.1	14.9
12	978	4.3	35.7	42	640	33.3	14.4
13	973	4.7	34.8	43	619	35.5	13.8
14	969	5.1	34.0	44	597	38.0	13.3
15	964	5.5	33.2	45	574	40.7	12.8
16	958	6.0	32.3	46	551	43.5	12.3
17	953	6.4	31.5	47	527	46.6	11.8
18	947	6.9	30.7	48	502	49.8	11.3
19	940	7.4	29.9	49	477	53.2	10.9
20	933	7.9	29.2	50	452	56.9	10.4
21	926	8.5	28.4	51	426	60.8	10.0
22	918	9.0	27.6	52	400	65.0	9.6
23	910	9.6	26.9	53	374	69.5	9.2
24	901	10.3	26.1	54	348	74.2	8.8
25	892	10.9	25.4	55	322	79.2	8.4
26	882	11.7	24.6	56	297	84.5	8.0
27	872	12.4	23.9	57	272	90.2	7.7
28	861	13.3	23.2	58	247	96.2	7.4
29	849	14.1	22.5	59	223	102.5	7.0
30	837	15.1	21.8	60	200	109.2	6.7

TABLE XVII (Continued)

LIFE TABLE FOR DROSOPHILA — WILD TYPE. LINE 107 — MALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
61	179	116.3	6.4	76	9	276.8	3.2
62	158	123.8	6.1	77	6	291.5	3.1
63	138	131.7	5.9	78	4	306.7	2.9
64	120	139.9	5.6	79	3	322.5	2.8
65	103	148.8	5.3	80	2	338.7	2.6
66	88	157.9	5.1	81	1	355.5	2.4
67	74	167.6	4.9	82	1	372.7	2.2
68	62	177.7	4.7				
69	51	188.3	4.4				
70	41	199.4	4.2				
71	33	211.0	4.1				
72	26	223.1	3.9				
73	20	235.8	3.7				
74	15	248.9	3.5				
75	12	262.6	3.4				

TABLE XVIII

LIFE TABLE FOR DROSOPHILA — WILD TYPE. LINE 107 — FEMALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.6	48.0	31	825	12.5	24.1
2	999	1.1	47.1	32	814	13.1	23.4
3	998	1.5	46.1	33	804	13.7	22.7
4	997	2.0	45.2	34	793	14.4	22.0
5	995	2.5	44.3	35	781	15.2	21.3
6	992	2.9	43.4	36	769	16.0	20.6
7	990	3.3	42.5	37	757	17.0	19.9
8	986	3.7	41.6	38	744	18.0	19.3
9	983	4.2	40.8	39	731	19.1	18.6
10	978	4.5	39.9	40	717	20.3	17.9
11	974	4.9	39.1	41	702	21.7	17.3
12	969	5.3	38.3	42	687	23.1	16.6
13	964	5.7	37.5	43	671	24.8	16.0
14	959	6.0	36.7	44	655	26.6	15.4
15	953	6.4	35.9	45	637	28.5	14.8
16	947	6.7	35.2	46	619	30.7	14.2
17	940	7.1	34.4	47	600	33.0	13.6
18	934	7.4	33.6	48	580	35.6	13.0
19	927	7.7	32.9	49	560	38.4	12.5
20	920	8.0	32.1	50	538	41.4	11.9
21	912	8.4	31.4	51	516	44.7	11.4
22	905	8.7	30.6	52	493	48.3	10.9
23	897	9.1	29.9	53	469	52.3	10.4
24	889	9.4	29.1	54	444	56.5	9.9
25	880	9.8	28.4	55	419	61.1	9.5
26	872	10.2	27.7	56	394	66.1	9.0
27	863	10.6	27.0	57	368	71.4	8.6
28	854	11.0	26.2	58	341	77.3	8.2
29	844	11.5	25.5	59	315	83.4	7.8
30	835	12.0	24.8	60	289	90.2	7.4

TABLE XVIII (Continued)

LIFE TABLE FOR DROSOPHILA — WILD TYPE. LINE 107 — FEMALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
61	263	97.3	7.0	76	16	278.3	3.2
62	237	105.0	6.6	77	11	295.9	3.0
63	212	113.2	6.3	78	8	314.4	2.8
64	188	122.0	6.0	79	6	333.4	2.7
65	165	131.3	5.7	80	4	353.2	2.5
66	144	141.3	5.4	81	2	373.6	2.4
67	123	152.0	5.1	82	1	394.7	2.2
68	105	163.2	4.8	83	1	416.6	1.9
69	87	175.1	4.6				
70	72	187.7	4.4				
71	59	201.0	4.1				
72	47	215.0	3.9				
73	37	229.7	3.7				
74	28	245.2	3.5				
75	21	261.3	3.3				

TABLE XIX

LIFE TABLE FOR DROSOPHILA — VESTIGIAL — MALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.0	14.1	26	72	162.7	5.8
2	1000	9.0	13.1	27	61	162.5	5.7
3	991	18.1	12.2	28	51	162.0	5.6
4	973	27.4	11.7	29	43	161.1	5.5
5	946	36.7	10.7	30	36	160.8	5.4
6	912	45.8	10.1	31	30	160.7	5.3
7	870	55.4	9.6	32	25	161.5	5.1
8	821	64.7	9.1	33	21	163.6	4.9
9	768	73.8	8.6	34	18	167.7	4.6
10	712	82.8	8.2	35	15	174.5	4.4
11	653	91.5	7.9	36	12	184.8	4.1
12	593	100.0	7.6	37	10	198.5	3.8
13	534	108.1	7.3	38	8	219.6	3.4
14	476	115.9	7.0	39	6	246.0	3.1
15	421	123.1	6.8	40	5	279.6	2.8
16	369	129.9	6.7	41	3	320.9	2.5
17	321	136.2	6.5	42	2	370.4	2.3
18	277	141.8	6.4	43	1	427.7	2.0
19	238	146.8	6.3	44	1	491.9	1.7
20	203	151.2	6.2				
21	172	154.8	6.1				
22	146	157.8	6.0				
23	123	160.0	6.0				
24	103	161.5	5.9				
25	86	162.4	5.9				

It is desirable to compare these *Drosophila* life tables with each other and with the human tables by putting the ages upon a relative base, using as a unit a centile (a hundredth part) of the equivalent life span, in the manner described in the text. The data of Tables 17 to 20 are transferred to a centile age basis in Table 21.

TABLE XX

LIFE TABLE FOR DROSOPHILA — VESTIGIAL — FEMALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	7.7	19.8	36	88	124.9	7.1
2	992	11.0	19.0	37	77	127.6	6.9
3	981	14.3	18.2	38	67	130.3	6.8
4	967	17.7	17.4	39	58	133.1	6.7
5	950	21.2	16.7	40	50	135.8	6.5
6	930	24.7	16.1	41	44	138.6	6.4
7	907	28.2	15.5	42	38	141.4	6.3
8	882	31.7	14.9	43	32	144.3	6.1
9	854	35.3	14.3	44	28	147.3	6.0
10	824	38.8	13.8	45	24	150.4	5.9
11	792	42.4	13.3	46	20	153.6	5.7
12	758	46.0	12.9	47	17	157.0	5.6
13	723	49.6	12.5	48	14	160.7	5.4
14	687	53.2	12.1	49	12	164.7	5.3
15	651	56.8	11.7	50	10	169.0	5.1
16	614	60.4	11.3	51	8	173.6	5.0
17	577	64.0	11.0	52	7	178.7	4.8
18	540	67.6	10.7	53	6	184.3	4.7
19	503	71.1	10.4	54	5	190.5	4.5
20	467	74.7	10.1	55	4	197.3	4.3
21	432	78.1	9.8	56	3	204.9	4.1
22	399	81.6	9.6	57	2	213.3	3.9
23	366	85.0	9.3	58	2	222.6	3.7
24	335	88.4	9.1	59	1	232.9	3.5
25	305	91.7	8.9	60	1	244.3	3.2
26	277	95.0	8.7	61	1	256.8	2.9
27	251	98.2	8.5				
28	226	101.4	8.3				
29	203	104.5	8.1				
30	182	107.5	8.0				
31	163	110.5	7.8				
32	145	113.5	7.6				
33	128	116.4	7.5				
34	113	120.1	7.3				
35	100	122.2	7.2				

TABLE XXI

SURVIVORSHIP DISTRIBUTIONS OF DROSOPHILA BY CENTILES OF LIFE SPAN

Centiles of Equivalent Life Span	Line 107		Vestigial		Centiles of Equivalent Life Span	Line 107		Vestigial	
	♂	♀	♂	♀		♂	♀	♂	♀
0	1000	1000	1000	1000	30	890	875	486	508
1	1000	1000	1000	996	31	882	868	462	487
2	999	999	1000	991	32	874	861	439	466
3	999	998	998	984	33	865	854	416	445
4	998	996	994	976	34	856	846	393	425
5	997	994	989	968	35	847	838	371	405
6	996	992	982	958	36	837	830	350	386
7	994	990	973	947	37	827	822	330	367
8	992	988	963	935	38	816	814	311	348
9	990	985	951	922	39	805	805	292	330
10	988	982	938	909	40	794	796	274	312
11	985	979	923	894	41	782	787	257	295
12	982	975	907	878	42	769	777	240	278
13	979	971	889	861	43	756	767	225	263
14	976	967	870	844	44	743	757	210	248
15	973	963	851	826	45	730	747	196	233
16	969	958	830	807	46	716	736	182	219
17	965	953	808	788	47	701	725	170	206
18	961	948	785	768	48	686	713	159	193
19	957	943	762	748	49	670	701	148	180
20	952	938	738	727	50	654	689	137	168
21	947	933	713	706	51	637	676	127	157
22	942	927	688	685	52	620	662	118	146
23	936	921	663	663	53	602	648	110	136
24	930	915	638	641	54	583	634	102	127
25	924	909	613	619	55	564	619	94	118
26	918	903	587	597	56	545	604	87	110
27	911	896	561	575	57	526	588	81	102
28	904	889	536	553	58	506	571	75	94
29	897	882	511	530	59	486	554	70	87

TABLE XXI (Continued)

SURVIVORSHIP DISTRIBUTIONS OF DROSOPHILA BY CENTILES OF LIFE SPAN

Centiles of Equivalent Life Span	Line 107		Vestigial		Centiles of Equivalent Life Span	Line 107		Vestigial	
	♂	♀	♂	♀		♂	♀	♂	♀
60	466	537	65	80	80	94	134	14	13
61	446	518	60	74	81	82	118	13	12
62	426	499	56	68	82	72	103	12	11
63	405	480	52	63	83	62	89	11	9.4
64	384	460	48	58	84	53	76	10	8.4
65	363	440	44	53	85	45	64	9.2	7.6
66	342	419	41	49	86	38	54	8.4	6.8
67	321	398	38	45	87	31	45	7.6	6.0
68	300	377	35	41	88	26	37	6.9	5.4
69	278	356	32	37	89	21	30	6.2	4.8
					90	17	24	5.5	4.2
70	258	334	30	34	91	14	19	4.8	3.7
71	240	312	28	31	92	11	15	4.2	3.2
72	221	290	26	28	93	8.3	11	3.7	2.8
73	202	269	24	26	94	6.4	8.3	3.2	2.4
74	184	248	23	24	95	4.8	6.1	2.7	2.1
75	167	227	21	22	96	3.6	4.5	2.3	1.8
76	150	207	19	20	97	2.6	3.2	1.9	1.6
77	135	188	18	18	98	2.0	2.2	1.6	1.4
78	120	169	17	16	99	1.4	1.4	1.3	1.2
79	106	151	15	14	100	1.0	1.0	1.0	1.0

2.

NUMERICAL DATA REGARDING THE INHERITANCE OF DURATION OF LIFE IN DROSOPHILA

THE following tables are taken from (106). They furnish the data from which the pedigree diagrams of Chapter V were prepared. The letters and numbers in the columns headed "Mating" and the numbers in the columns headed "Type" refer to the scheme of breeding shown as Table 7 of the text.

TABLE XXII

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF WILD TYPE F_2 FLIES FROM *inter se* MATINGS OF F_1

Mating	Type	Mean Age at Death	Standard Deviation	Coefficient of Variation
P × P (Total)	(5)	46.34 ± .73 days	15.29 ± .51 days	33.00 ± 1.22
P ♂ × Z ♀ (76)...	(3)	39.16 ± .83 days	13.26 ± .59 days	33.86 ± 1.67
Z ♂ × P ♀ (77)...	(3)	35.89 ± 1.01 days	14.78 ± .71 days	41.18 ± 2.30
P × Z (Total)	(3)	37.66 ± .65 days	14.08 ± .46 days	37.37 ± 1.38
Z × Z (Total)	(7)	46.01 ± .73 days	16.19 ± .52 days	35.19 ± 1.25
All *inter se* matings.	(5) + (3) + (7)	43.33 ± .42 days	15.75 ± .30 days	36.34 ± .77

TABLE XXIII

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF VESTIGIAL F_2 FLIES FROM *inter se* MATINGS OF F_1

Mating	Type	Mean Age at Death	Standard Deviation	Coefficient of Variation
P × P (Total)	(5)	13.10 ± .79 days	9.26 ± .54 days	70.70 ± 6.01
P ♂ × Z ♀ (76)...	(3)	17.45 ± 2.18 days	10.23 ± 1.54 days	58.64 ± 11.49
Z ♂ × P ♀ (77)...	(3)	18.81 ± .89 days	7.67 ± .63 days	40.78 ± 3.85
P × Z (Total)	(3)	18.50 ± .85 days	8.34 ± .60 days	45.09 ± 3.85
Z × Z (Total)	(7)	13.15 ± 1.31 days	12.46 ± .93 days	94.78 ± 11.81
All *inter se* matings.	(5) + (3) + (7)	14.60 ± .57 days	10.31 ± .40 days	70.65 ± 3.91

TABLE XXIV

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF WILD TYPE F_2 FLIES FROM BACK CROSSES

Matings	Type	Mean Age at Death	Standard Deviation	Coefficient of Variation
Wild ♂ × Z ♀ (80)	(8)	32.56 ± .83 days	14.67 ± .59 days	45.07 ± 2.14
Z ♂ × wild ♀ (81)	(8)	33.91 ± .93 days	18.19 ± .66 days	53.64 ± 2.43
Total Z × wild	(8)	33.30 ± .63 days	16.72 ± .45 days	50.20 ± 1.65
Wild ♂ × P ♀ (84)	(4)	36.57 ± .68 days	12.85 ± .48 days	35.13 ± 1.47
P ♂ × wild ♀ (85)	(4)	38.83 ± .82 days	17.31 ± .58 days	44.59 ± 1.77
Total P × wild	(4)	37.82 ± .55 days	15.52 ± .39 days	41.03 ± 1.18
Total back cross on wild.....	(8) + (4)	35.72 ± .42 days	16.25 ± .30 days	45.48 ± .99
Vestigial ♂ × Z ♀ (78).....	(2)	35.63 ± .91 days	10.81 ± .64 days	30.34 ± 1.97
Z ♂ × Vestigial ♀ (79).....	(2)	26.56 ± 2.11 days	15.29 ± 1.49 days	57.57 ± 7.23
Total Z × Vestigial	(2)	33.15 ± .92 days	12.85 ± .65 days	38.75 ± 2.25
Vestigial ♂ × P ♀ (82).....	(6)	32.52 ± 1.09 days	14.05 ± .77 days	43.22 ± 2.79
P ♂ × Vestigial ♀ (83).....	(6)	29.33 ± 1.05 days	11.46 ± .74 days	39.05 ± 2.90
Total P × Vestigial	(6)	31.19 ± .78 days	13.12 ± .55 days	42.08 ± 2.06
Total back cross of vestigial.....	(2) + (6)	31.98 ± .60 days	13.05 ± .42 days	40.80 ± 1.53

TABLE XXV

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF VESTIGIAL F_2 FLIES FROM BACK CROSSES

Mating	Type	Mean Age at Death	Standard Deviation	Coefficient of Variation
Vestigial ♂ × Z ♀ (78).....	(2)	16.47 ± .59 days	7.41 ± .42 days	45.01 ± 3.00
Z ♂ × Vestigial ♀ (79).....	(2)	11.41 ± .78 days	7.64 ± .55 days	66.92 ± 6.63
Total Z × Vestigial	(2)	14.55 ± .49 days	7.89 ± .35 days	54.22 ± 3.03
Vestigial ♂ × P ♀ (82).....	(6)	12.86 ± .42 days	5.20 ± .30 days	40.44 ± 2.66
P ♂ × Vestigial ♀ (83).....	(6)	10.33 ± .41 days	5.37 ± .29 days	52.06 ± 3.45
Total P × Vestigial	(6)	11.51 ± .30 days	5.44 ± .21 days	47.30 ± 2.22
Total back crosses on Vestigial	(2) + (6)	12.84 ± .28 days	6.79 ± .20 days	52.91 ± 1.93
Total F_1 ♂ × Vestigial ♀.....	—	10.71 ± .38 days	6.29 ± .27 days	58.75 ± 3.27
Total Vestigial ♂ × F_1 ♀.....	—	14.69 ± .38 days	6.67 ± .27 days	45.38 ± 2.16

TABLE XXVI

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF WILD TYPE F_3 FLIES

Mating	Type	Mean Age at Death	Standard Deviation	Coefficient of Variation
Z F_1 ♂ × Vest. D_{76} F_2 ♀ (86).	(54)	32.53 ± 1.22	15.74 ± .86	48.37 ± 3.21
Z F_1 ♂ × Vest. D_{77} F_2 ♀ (90).	(54)	24.68 ± .87	10.55 ± .61	42.80 ± 2.92
Total Z F_1 ♂ × Vest. D ♀....	(54)	28.85 ± .80	14.11 ± .56	48.91 ± 2.37
P F_1 ♂ × Vest. D_{76} F_2 ♀ (88).	(52)	27.25 ± 1.10	15.88 ± .78	58.29 ± 3.70
P F_1 ♂ × Vest. D_{77} F_2 ♀ (92).	(52)	31.40 ± 1.44	16.90 ± 1.02	53.83 ± 4.07
Total P F_1 ♂ × Vest. D ♀....	(52)	28.90 ± .88	16.42 ± .62	56.82 ± 2.77
P F_1 ♂ × Wild type D_{76} F_2 ♀ (89).....	(52)	32.05 ± .72	14.18 ± .51	44.26 ± 1.88

TABLE XXVII

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF VESTIGIAL F_3 FLIES

Mating	Type	Mean Age at Death	Standard Deviation	Coefficient of Variation
Z F_1 ♂ × Vest. D_{76} F_2 ♀ (86).	(54)	5.28 ± .28	4.64 ± .20	87.95 ± 6.09
Z F_1 ♂ × Vest. D_{77} F_2 ♀ (90).	(54)	7.07 ± .33	4.21 ± .23	59.60 ± 4.26
Total Z F_1 × Vest. D ♀.....	(54)	5.97 ± .22	4.57 ± .16	76.49 ± 3.83
P F_1 ♂ × Vest. D_{76} F_2 ♀ (88).	(52)	9.93 ± .45	6.98 ± .32	70.26 ± 4.57
P F_1 ♂ × Vest. D_{77} F_2 ♀ (92).	(52)	8.17 ± .28	4.52 ± .20	55.29 ± 3.07
Total P F_1 ♂ × Vest. D ♀...	(52)	9.00 ± .26	5.88 ± .19	65.30 ± 2.82

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